PREDATOR BIOMASS AND HABITAT CHARACTERISTICS AFFECT THE MAGNITUDE OF CONSUMPTIVE AND NON-CONSUMPTIVE EFFECTS (NCEs): EXPERIMENTS BETWEEN BLUE CRABS, MUD CRABS, AND OYSTER PREY

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PREDATOR BIOMASS AND HABITAT CHARACTERISTICS AFFECT THE MAGNITUDE OF CONSUMPTIVE AND NON-CONSUMPTIVE EFFECTS (NCEs): EXPERIMENTS BETWEEN BLUE CRABS, MUD CRABS, AND OYSTER PREY

Approved By:

Dr. Marc Weissburg, Advisor
School of Biology
Georgia Institute of Technology

Dr. Mark Hay
School of Biology
Georgia Institute of Technology

Dr. Julia Kubanek
School of Biology
Georgia Institute of Technology

Dr. Todd Streelman
School of Biology
Georgia Institute of Technology

Dr. Jeb Byers
Odum School of Ecology
University of Georgia- Athens

Date Approved: June 30, 2011

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Summary

In recent years, increasing attention has been focused on the non-lethal effects of predator intimidation and fear, dubbed non-consumptive effects (NCEs), in which prey actively change traits, such as behavior and habitat use, in response to predator cues. NCEs can propagate a wide variety of indirect interactions that influence community structure and often are mediated by predator chemical cues in aquatic communities (Lima, 1998; Kats and Dill 1998; Werner and Peacor, 2003). Further, the strength of NCEs is often context dependent upon the risk perceived from predators, as well as the behavioral and evolutionary ecology of the prey (Ferrari et al. 2010); thus, current research seeks to understand both the predatory and environmental contexts in order predict when NCEs are likely to be important structuring forces in communities and to further understand interaction strength between species through NCEs.

The structure of natural communities and habitats present a number of factors that are likely to affect the magnitude of NCE including predator population structure and the sensory landscape of the natural environment. Intraspecific variation in predator populations such as body size, density, and distribution may affect the assessment of predator risk and thus the outcome of NCEs. Since body size is often an indication of risk in gape or crush limited predators (Werner and Gilliam 1984), predator body size will likely affect whether predators are ‘risky’ and consequently may mediate risk perception and antipredator behaviors (Helfman, 1989). In the context of chemical cues, predator body size may be perceived through differing cues or via cue concentration from differing biomasses of predators (Chivers et al. 2001; Kusch et al. 2004). The latter

suggests that the density and aggregation of predators may be an important factor in determining predator risk as prey may be unable to differentiate large from many small animals on the basis of chemical cues alone. In contrast, individual predator size may be easily distinguished based on visual cues (Stankowich and Blumstein, 2005) suggesting that the interpretation of predator risk via biomass maybe highly dependent upon cue type. However, current methods of characterizing NCEs rarely use more than one size class of predator much less investigate interactions of predator density and size using differing sensory modalities.

The sensory landscape of the environment, or the limitations the environment poses on sensory modalities (e.g. habitat complexity, turbidity etc.), will often restrict the information that prey can perceive from predators as well as the number of modalities by which risk is determined (Hartman and Abrahams 2000; Powers and Kittinger 2002; Smee et al. 2008). This context is important as the number of cues that prey perceive likely increases prey certainty and can contribute to threat assessment. Consequently, the sensory landscape can dictate the magnitude of NCE in two ways: 1) by decreasing the detection of risk through degradation of cues that propagate NCEs and; 2) by increasing prey uncertainty about predator risk by limiting the number of sensory cues available.

Further, the sensory landscape that is representative of natural environments is rarely replicated in NCE studies, especially in aquatic environments. Aquatic studies often use laboratory mesocosms in which chemical cues move by diffusion and can persist for long periods (Ferrari et al. 2010). In natural environments, properties such as high velocity and turbulence quickly disperse chemical cues and consequently can interfere with chemical cue perception and the outcome of NCEs (Weissburg 2000; Smee et al. 2008,

Smee et al. 2010). Thus, current studies may overestimate the magnitude and prevalence of NCEs by not replicating natural conditions where cue perception can be inhibited. Further, as flow can vary over temporal and spatial scales, NCEs may also vary spatially and temporally in the field suggesting that monitoring flow conditions in space and time may be necessary to explain variability in NCEs (Smee et al. 2010). By encompassing natural variation in NCEs, we can better predict conditions under which these interactions can significantly structure communities. However, few studies have simultaneously measured flow and NCEs or used temporally varying profiles as in the field.

In order to ascertain the role of predator size, density, sensory information, as well as the natural flow environment in the magnitude of NCEs, I performed a series of both lab and field experiments in an intraguild predation system composed of both adult and juvenile blue crab predators (*Callinectes sapidus*), mud crabs (*Panopeus herbstii*) and their shared oyster prey (*Crassostrea virginica*). In order to understand the consumptive interactions between these species, I performed multiple experiments examining the importance of blue crab predator size and habitat type on mud crab survivorship, as well as the abilities of each predator to consume shared oyster resources. In laboratory experiments, both medium and small size classes of blue crabs had relatively little effect on mud crab survivorship, regardless of habitat type. In contrast, large blue crabs had high predation rates on mud crabs, but these effects were mitigated by increasing habitat complexity whereby oyster reef habitats served as a refuge from predation. The role of oyster reefs as a predation refuges was confirmed the field using tethering experiments. These experiments demonstrated that large blue crabs are an important source of mud crab mortality and provided a basis for mud crab risk assessment whereby large blue crabs are

risky and small blue crabs are not. Further, blue crabs were less likely to consume oysters in lab predation experiments in comparison with mud crabs that consumed oysters at high rates suggesting that trophic cascades may occur in this system when mud crabs are behaviorally suppressed or consumed.

In order to determine whether risk associated with predator size is perceptible through chemical cues and is related to predator biomass, I investigated the behavioral response of mud crabs and their predation rates on oysters in response to differing biomasses of caged blue crab predators. Mud crab predation on oysters was decreased in response to high biomass treatments (i.e. large and multiple small blue crabs), but not to low biomass predators (i.e single small blue crab), suggesting that risk associated with predator size is perceptible via chemical cues and is based on predator biomass. However, mud crab activity was only suppressed in response to large blue crabs. Thus, multiple small blue crabs caused differential behaviors that resulted in less foraging, but not decreased activity. As multiple sensory stimuli may have affected responses to many small blue crabs, experiments were repeated using only chemical cues. Mud crabs responses did not differ between large and many small predators via only chemical cues supporting the hypothesis that multiple sensory cues caused differing behaviors. Further experiments also demonstrated that predator diet affected mud crab antipredator behavior. To my knowledge this study is the first to show that differences in predator body size and density can cause differing indirect NCEs and that these differences are mediated by the concentration of chemical cues associated with predator biomass. Consequently, current studies may misestimate the magnitude of NCE by assuming all predators, regardless of size and distribution, produce the same effects. It also demonstrates prey activity level is

not always predictive of the impact on lower resource levels due to the sensory contexts under which risk is determined. Furthermore, considering only one sensory modality, or one diet, in NCEs may affect the prey's perception of risk and result in differing context dependent outcomes for both NCEs and community structure.

Although cues associated with predator biomass are perceptible in laboratory mesocosms, water flow in the natural environment distributes cues as odor plumes where the concentration of cues is often patchy and unpredictable (Weissburg 2000). This suggests the perception of predator body biomass via chemical cue concentration in natural environments may be problematic as properties of the physical environment, such as high velocity and turbulence, can quickly disperse cues and interfere with cue perception. Thus, I examined the foraging behavior of mud crabs in response to differing biomasses of blue crab predators under natural conditions using field caging experiments. As in lab experiments, cues from high predator biomass treatments suppressed mud crab foraging on oysters in field cages. Foraging in response to low biomass predators was similar to zero crab controls. During these experiments, acoustic Doppler velocimeters (ADVs) were used to characterize the flow environment and verified that NCEs took place under realistic physical conditions. The presence of flow and turbid field conditions did not dampen the NCE of blue crabs in oyster reef habitats and any differences in predation overtime were not associated with local flow parameters. Thus, predator biomass can affect the magnitude of NCEs in natural environments and both predator body size and distribution will be important to determining the role of NCEs in ecological networks. These experiments provide a positive link between blue crab and oyster populations, both highly important commercial fisheries. Furthermore, blue crabs are among several

commercial species currently undergoing shifts to a smaller mean body size (Lipcius and Stockhausen 2002). My data suggests that fisheries that alter predator size structure will likely alter the NCEs they propagate in communities.

Finally, although previous experiments demonstrate that interactions between crab predators are likely important for oyster survival, it is unclear whether these short term interactions are predictive of long term outcomes on oyster reefs (Werner and Peacor 2003). Thus, I investigated the influence of mud crabs and blue crab chemical cues on the long term successful recruitment and growth of oysters in the field to determine if interactions between these species were important for oyster survival and reef development. Oyster recruitment in the field was extremely high and effectively swamped mud crab predators. As such, evidence of predation or a NCE of blue crab chemical cues was minimal; although, this may be attributable to complications in experimental design. However, both mud crab treatments significantly modified oyster size class distributions through suppression of oyster growth suggesting that the presence of mud crabs can increase the time period when oysters are vulnerable to predation and other sources of juvenile mortality. Consequently, longer term experiments may be able to determine the full consequences of mud crab predators to oyster reef communities. Although the conclusions that predation and NCEs can impact oyster recruitment are mixed, this study represents a first attempt to understand both the role of predation and NCEs of predator cues on post-settlement mortality of oysters.

In conclusion, these studies which demonstrate intraspecific predator variation and sensory landscape can alter prey antipredator behavior and NCEs have broad consequences for both the management of estuarine fisheries, conservation, and ecology.

Although recent research has championed the role of NCE in communities (Preisser et al. 2005), much of this research has not considered the natural variation in communities that can affect NCEs. By ignoring intraspecific variation in predator populations and assuming all predators cause equal effects, we are likely misestimating the role of NCEs in communities. As population size structure is an important aspect of predicting NCEs, future conservation efforts should focus on not only protecting species, but also preserving the species population structure. Commercial fisheries often modify species population structure by selectively removing larger individuals and blue crabs are among species whose population structure has been altered due to overfishing (Lipcius and Stockhausen 2002). This anthropogenic impact is likely affecting both the consumptive and NCEs which can increase oyster survival (Shackell et al. 2009). In addition, the information and sensory modalities by which prey assess risk can affect the strength and direction of prey behavior and result in differing predictive outcomes for indirect NCEs. This demonstrates that prey behavior is not sufficient to predict community level interactions. The role of the sensory landscape in mediating NCEs dictates performing these studies in the context of the natural environment. Results from my field study suggest that NCE research can be performed in the field with minimal impacts to flow parameters. Further, field results were similar to the lab revealing that NCEs occur in estuarine field environments. Consequently, NCEs are likely important structuring forces in estuarine environments; although, it is still likely that flow can affect the outcome of NCEs in estuarine communities over a wider range of flow regimes (Smee et al. 2010).

CHAPTER ONE

Resource use, habitat complexity, and predator size mediate interactions between intraguild blue crab predators and mud crab prey

Abstract

Despite theoretical predictions that intraguild predation (IGP) should be unstable, intraguild (IG) predators and prey coexist in a wide variety of systems. The persistence of IGP in natural environments is likely a product of resource use, habitat refuges, and predator and prey population structure that are often not incorporated into simplistic IGP models. Here, I investigated the role of resource use, predator size, and habitat complexity in mediating the interactions of IG predators, blue crabs (*Callinectes sapidus*), and IG prey, mud crabs (*Panopeus herbstii*) in oyster reef communities. Mud crabs consistently consumed oysters at higher rates than blue crabs demonstrating that mud crabs are the greater consumer of shared oyster resources. In laboratory mesocosms, oyster reefs provided a habitat refuge from blue crab predation suggesting that mud crabs gain a refuge from IG predators with increasing habitat complexity. The survivorship of mud crabs in less complex habitats was significantly dependent on predator size, where less efficient small blue crab predators were unable to successfully consume mud crabs in any habitat type. Furthermore, the refuge effects of oyster reef were confirmed in the field with tethered mud crabs providing field evidence that oyster reefs provide refuges for crab inhabitants. These experiments demonstrate the importance of habitat complexity and body size in mediating IGP in natural systems as well as elucidating the impacts of blue crabs and mud crabs in ecologically and economically valuable oyster reef communities.

Introduction

Intraguild predation (IGP) is defined as predation between species that also share a common prey (Polis et al. 1989, Polis and Holt 1992, Holt and Polis 1997). In its simplest form, IGP involves three species: an intraguild (IG) predator that consumes an IG prey, while both species compete for a common shared resource. Theoretical predictions often determine that IGP should be uncommon in nature due to its instability (Polis and Holt 1992, Holt and Polis 1997, Mylius 2001). In particular, IG prey who must compete for shared resources and can be eaten by their potential competitors are likely to be driven to extinction (Polis et al. 1989, Polis and Holt 1992, Mylius 2001). Coexistence of IG prey and IG predators is only predicted if the IG predator is a relatively poor competitor and inefficient at feeding on the common resource (Polis and Holt 1992, Mylius et al. 2001). However, despite these theoretical predictions, IGP is a widespread interaction in nature (Polis et al. 1989, Polis and Holt 1992, Arim and Marquette, 2004).

The contradiction between theory and reality in IGP is likely due to the simplistic nature of IGP models that often do not incorporate components of natural habitats or variance in predator and prey population structure that frequently mediate interactions between IG predators and prey in field environments. For instance, many previous studies have documented that habitat complexity can serve as a refuge from predation for a multitude of organisms (Heck and Thoman 1981, Crowder and Cooper 1982, Diehl 1992, Persson and Eklov 1995). These refuge effects result from reduced predator foraging efficiency through increased predator search time, reduced prey encounters, or inhibited perception of prey in complex habitats (Crowder and Cooper 1982, Eklov and Diehl 1994, Persson and Eklov 1995, Ferner et al., 2009). As such, the predation rates often decline with

increasing habitat complexity. Yet, the importance of habitat complexity in mediating interactions between IG predators and prey has been investigated only recently (Finke and Denno 2002, Janssen et al. 2007, Schmidt and Rypstra 2010). Evidence suggests that increased habitat complexity can facilitate the coexistence of IG predators and prey by reducing encounter rates (Finke and Denno 2002). For instance, increasing habitat complexity in arthropod terrestrial systems leads to decreased intraguild predation and cannibalism between spider predators, and increases suppression of shared grasshopper prey (Finke and Denno 2006). In addition, a meta-analysis of multiple IG predation studies has demonstrated that increased habitat complexity decreases predation rates of IG predators on IG prey (Janssen et al. 2007). Thus, habitat complexity may mediate interactions between IG predators and IG prey by providing a refuge to IG prey.

Additionally, ontogenetic changes in body size may create variance in effects of IG predator and IG prey over time by affecting both the trophic structure as well as competitive outcomes. Yet, body size of IG predators and prey is frequently unaddressed in theoretical models of IGP (but see Mylius et al. 2001, van de Wolfshaar et al., 2006) In natural communities, both predators and prey undergo ontogenetic changes in body size that can affect the outcomes of IGP predation (van de Wolfsharr et al. 2006, Rudolf and Armstrong 2008). Animal body size often dictates a number of predator traits including prey identity and consumption rates of organisms (Werner and Gilliam, 1984, Cohen et al. 1993). Further, body size can influence an animal's ability to compete with both conspecifics and heterospecifics (Menge, 1972; Wissinger, 1992). Thus, the body size of both IG predators and IG prey may facilitate IGP in nature by dictating competitive and predatory interactions in natural communities.

Although both predator size and habitat complexity can influence prey survivorship, the two factors are rarely considered simultaneously (but see Babbitt and Turner 1998). This paucity is significant as predator body size can affect the efficiency of predator interactions with habitat structure (Babbitt and Tanner 1998; Lewis and Eby 2002, Sarty et al. 2006) whereby smaller predators may be more apt to penetrate small interstitial spaces created by increased habitat complexity (McDonald 1982). For instance, body size often dictates a predator's ability to penetrate differing rugosity environments and provides an avenue for competitive coexistence (McDonald 1982, Sarty et al. 2006). In addition, smaller predator size classes may be less efficient consumers and habitat structure may only reduce predation rates of larger size classes (Babbitt and Turner 1998). Consequently, increased habitat structure may not confer the same refuge benefits from all predator size classes.

The goal of my study was to examine the effects of resource use, habitat complexity, and predator size in mediating the interactions between IG crab predators in oyster reef communities. Oyster reefs provide a variety of ecological services to estuarine communities (Newell 2004) including serving as habitat for a multitude of species such as various crab predators and bivalve species (Wells 1961). Due to the diverse assemblages of prey, oyster reefs may attract a number of predator species including blue crabs, whelks, and demersal fishes (Langelletto and Denno 2004, Wilson and Weissburg *unpublished manuscript*). As such, previous studies have demonstrated that the increased structural complexity of oyster reefs mediates a number of predator-prey interactions within them (Micheli and Peterson 1999, Grabowski 2004, Hughes and Grabowski 2006, Grabowski 2008). For instance, structurally complex oyster reefs reduce interference

interactions among both conspecific and heterospecific predators (Grabowski and Powers 2004, Hughes and Grabowski 2006). However, the importance of oyster reef structure in mediating prey survival has rarely been demonstrated in the field and to my knowledge has only been demonstrated for bivalve species (Micheli and Peterson 1999) and porcelain crabs (*Petrolisthes armatus*; Hollebone and Hay 2008).

Due to their diverse species assemblage, oyster reefs are inhabited by multiple guilds of predators that feed at differing trophic levels. Among these species, I chose to investigate the IG predator, the blue crab (*Callinectes sapidus*) and IG prey, mud crabs (*Panopeus herbstii*). Blue crabs are important predators in estuarine communities (Micheli 1997) and prey on a variety of crustacean and bivalve species including oysters (*Crassostrea virginica*; Fitz and Weigert 1991, Micheli, 1997, Eggleston 1990). Juvenile blue crabs recruit to structurally complex habitats such as oyster reefs (Mosknes and Heck 2006) and adult blue crabs readily track to chemical cues emanating from reefs (Wilson and Weissburg *unpublished manuscript*). However, there are few studies documenting the predatory interactions of blue crabs within multispecies oyster reefs (but see O'Connor et al. 2008 and Grabowski et al. 2008). Mud crabs are xanthid crabs which occupy oyster reefs at high densities and consume a number of bivalve species including mussels and oysters (Seed 1980, Bisker and Castagna 1987). Although xanthid and other non-portunid crabs make up approximately 43% percent of blue crab diets in gut content analysis (Fitz and Weigert 1991), experiments demonstrating predatory interactions between the two species are limited (but see Grabowski et al. 2008) and it is unknown which body sizes of blue crabs are able to consume mud crabs. Furthermore, there are few demonstrations of which crab species or size classes best utilize oyster prey (but see Grabowski et al. 2008),

especially when oyster prey are located in complex habitat structure. As oysters are ecosystem engineers which provide a variety of ecosystem services to estuarine habitats (reviewed by Newell 2004), both blue crab and mud crab consumption of oysters may have significant impacts to estuarine ecosystems.

Based on these unknowns in this system, I wanted to investigate the role of structural complexity provided by oyster reefs, as well as predator body size, in the intraguild predation interactions between blue and mud crabs. The goals of my study were to: a) determine which predator, mud crabs or blue crabs, were the greater consumer of oysters; b) document the predatory interaction between blue crabs and mud crabs; c) investigate the role of blue crab predator size and habitat type on mud crab predation; d) to confirm that mechanisms mediating IGP observed in the lab also operate in natural field environments.

Methods

Animal collection and maintenance

Experiments were performed at the Skidaway Institute of Oceanography (SkIO), Skidaway Island, Georgia, USA. Both blue crabs and mud crabs were collected from Wassaw Sound and associated tributaries. Blue crabs were collected by commercial crab pot and seine net. Mud crabs were collected by hand from loose oyster reef. Oysters (10-20mm in length) were obtained from Bay Shellfish (Tampa, FL). All animals were maintained in covered outdoor flow-through seawater tanks at SkIO for a minimum of 48 hours before experiments began. Blue crabs were maintained on a diet of shrimp and/or clams and were starved 24 hours prior to experiments. Mud crabs were maintained on a

clam diet and were starved for 24 hours prior to oyster consumption experiments and fed prior to blue crab predation experiments to limit cannibalism.

Use of a shared resource by mud crabs and blue crabs

In order to determine which predator was the best consumer of oyster prey, I defined the best consumer as the predator that could reduce the abundance of the shared prey to the lowest abundance in the absence of the other predator. This measurement is often used as a proxy for competitive ability in IGP models (Polis and Holt 1992, Holt and Polis 1997). I then compared predation rates on oysters in laboratory mesocosms between mud crabs (25-45mm carapace width; CW) and two size classes of blue crab predators (>100mm CW; 60-80mm CW). Two size classes of blue crab predator were used as blue crab body size may affect the ability of crabs to access oyster prey within complex reef structure.

Predation on oysters by each predator was examined on mesocosms containing approximately 2.5 cm of sand and artificial oyster reef that mimicked the complex structure of oyster reef habitat. Artificial reef was used to control for placement of live oysters within reefs and was constructed by gluing 10 oyster shells (obtained from natural shell banks) to create similar small clusters (approximately 6 cm in diameter). A total of 13 clusters were used to build artificial reefs. Seven of these artificial clusters had three juvenile oysters (one of each size class length: 15-16mm, 16-17mm, 17-18mm) glued to its face with cyanoacrylate glue (21 oysters total). Mud crabs and blue crabs have been shown to feed on oysters between 15-20mm in length (Bisker and Castagna 1987, Eggleston 1990b). Artificial clusters with oysters were then secured to a second artificial cluster using rubber bands and placed around a center oyster cluster to create a reef-like structure. Artificial oyster clusters were arranged so that live juvenile oysters faced both

into and out of the reef in order to control for edge effects. One of each predator treatment was placed within artificial mesocosm reefs in a randomized block design. I monitored the number of oysters consumed by each predator every 24 hours for 72 hours. Five replicates were run at one time for two experimental runs, a total of 10 replicates. Preliminary data analysis showed that run (time) did not significantly affect the number of oysters eaten; consequently, all data from runs were pooled. Since data could not be transformed to meet assumptions of normality, the number of oysters eaten at the completion of the experiment was examined by a one-way nonparametric Kruskal-Wallis (K-W) for the effects of predator type (Large blue crab, medium blue crab, mud crab). I excluded any replicate where the predator died or molted during the course of the experiment.

Blue crab predation on mud crabs mediated by body size and habitat

I performed a 3x3 factorial experiment in a randomized block design in which I manipulated blue crab predator size (>100 mm CW, 60-80mm CW, 40-60mm CW) and habitat complexity (oyster reef, shell hash, and sand) in order to examine the effects of predator size and habitat complexity on mud crab survivorship. Differing habitat types were constructed in laboratory mesocosms which consisted of covered outdoor fiberglass tanks (0.62m x 0.50m x 0.27m) supplied with flow-through seawater. All mesocosms contained approximately 2.5 cm of sand, which was the lowest habitat complexity used in experiments (sand only). Oyster reef and shell hash habitats were constructed on top of sand substrates. Shell hash habitats were constructed by scattering approximately two liters of oyster shell hash (obtained from natural shell banks) in the center of mesocosms. Oyster reef habitats consisted of artificial oyster clusters (similar to previous experiment)

and then bundling clusters with rubber bands to build a reef in the center of mesocosms (4 cm in diameter; 27 clusters per tank). These artificial reefs allowed us to maintain a homogenous reef structure across tanks.

Ten mud crabs (similar to natural size distributions seven 15-20mm CW, two 20-25mm CW, one 25-30mm CW; Lee and Kneib 1994) were added to mesocosms containing each structure type and allowed to acclimate to tank habitats overnight. One of three size classes of blue crabs was then assigned randomly to each habitat type and placed in mesocosms. Blue crabs were allowed to forage on mud crabs for approximately 18 hours that encompassed both the evening and overnight hours when both mud crabs and blue crabs are most active (Clark et al. 1999, Grabowski 2004). No-predator controls consisting of ten mud crabs in sand habitats were also performed at the same time as predation trials in order to determine mud crab background mortality. The number of mud crabs surviving in all treatments and controls was recorded at the conclusion of the experiment. Replicates were excluded where the blue crab died or molted. Two replicates of each treatment were performed simultaneously due to limited tank space. This was repeated for three experimental runs totaling six replicates of each treatment. Due to deaths and molting, another experimental run consisting of only 40-60mm CW crabs and >100mm CW crabs was added at the end of the experiment. The distribution of surviving mud crabs was non-normal despite a variety of transformations. However, as ANOVAs are typically robust for non-normal distributions (Zar 1999), I completed the analysis using a two-way ANOVA for the effects of predator size and habitat type. I confirmed significance for habitat type and predator size using two one-way nonparametric Kruskal-Wallis (K-W) tests.

Investigating oyster habitat refuge effects in the field

The previous experiment demonstrated that increasing habitat complexity increased mud crab survivorship rates in laboratory mesocosms. In order to confirm this pattern in the field, I performed a tethering experiment in which I assessed predation rates on tethered mud crabs in differing habitats. Predation rates measured by tethering animals are often difficult to interpret as tethers often restrict escape behaviors that limit predation (Zimmer et al. 1994, Aronson and Heck 1995). However, mud crabs typically rely on their cryptic nature and proximity to refuge habitat for predator defense (Hill, *personal observations*), suggesting that tethering may be a relatively accurate measure of predation for this species. The tethering experiment was performed at two different sites in Wassaw Sound, GA: Priest's Landing (PL) and Dead Man's Hammock (DMH). Both sites are characterized by patches of loose and fixed oyster reef interspersed with shell hash and sand/mud habitat. Mud crabs as well as a suite of potential mud crab predators including blue crabs and demersal fishes are common to both locations (Hill, *observations*).

I examined mud crab survival by tethering 30 mud crabs in each of three differing habitat types: oyster reef, shell hash, and bare habitats. Tethers consisted of monofilament line (~25cm, 10lb test) that was tied around mud crabs (20-25mm CW) between the chelipeds and legs and secured to the top of the carapace with cyanoacrylate glue. The other end of the tether was secured to a landscaping stake. Each stake was then numbered and labeled with orange flagging tape so that the survival of individuals could be tracked. Tethered mud crabs were maintained overnight in flow through seawater tanks for a minimum of 12 hours where less than 3% died or escaped their tethers. Mud crabs were then transported into the field in coolers where 30 mud crabs were secured in the substrate in

each habitat type. Each tethered animal was approximately 1-2 meters apart in each habitat type and was marked with a survey flag for ease of retrieval. Tethers were monitored for the presence or absence of mud crabs at 24 hours. The number of mud crabs remaining in each habitat type at each site was then analyzed by Chi-square.

Results

Use of a shared resource by mud crabs and blue crabs

Blue crabs and mud crabs significantly differed in their ability to consume juvenile oysters (Fig.1.1; K-W, H= 17.16, DF=2, P<0.001). Mud crabs consumed approximately 15 oysters on average over the course of the experiment. However, neither size class of blue crabs was a significant consumer of oysters, consuming on average only one oyster during the experimental period (Fig.1.1)

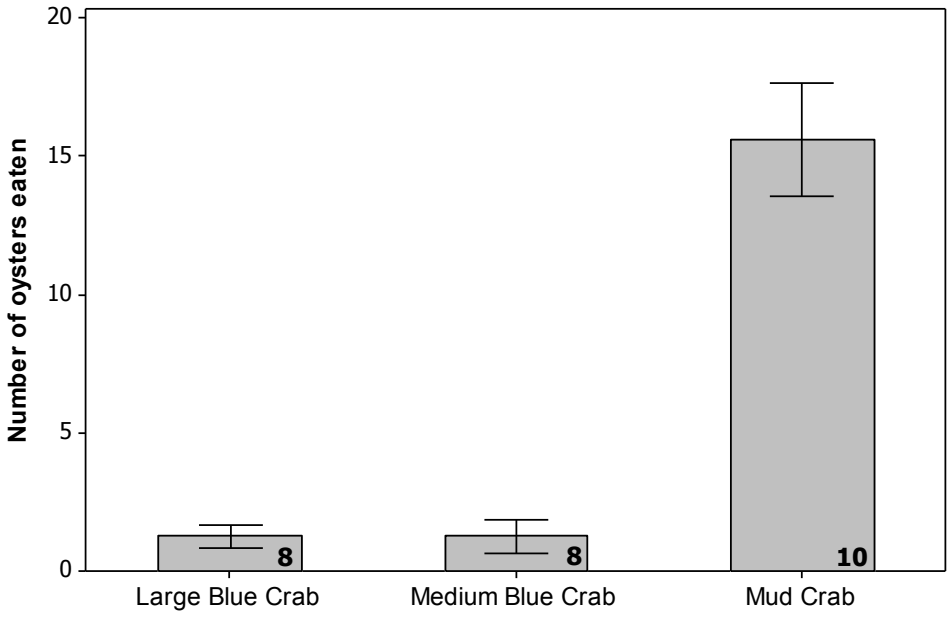

Figure 1.1. Predation on oysters (mean ± SE) by differing predators. Mud crabs consumed significantly more oysters than blue crabs of either size class ($F_{2,20}$=31.02, P<0.001). Numbers at the base of graph bars denote number of replicates. 21 oysters were secured to reefs in each mesocosm.

Blue crab predation on mud crabs mediated by body size and habitat

Mud crab survivorship was significantly affected by both predator size (Fig.1.2; ANOVA, $F_{2,50}$=29.34, P<0.001; K-W, P<0.002) and habitat type (ANOVA, $F_{2,50}$=18.73, P<0.001;K-W P < 0.03), but the magnitude of the effect was dependent upon the combination of each level (Predator Size × Habitat; ANOVA, $F_{4,50}$=13.22,P<0.001). Large blue crabs (>100mm CW) were efficient predators of mud crabs consuming almost all mud crabs in sand habitats (Fig.1.2). However, mud crab survivorship increased with habitat complexity, reaching a maximum of almost 90% in reef habitats. Medium sized blue crabs (60-80mm CW) were inefficient predators relative to large blue crabs; approximately 6-7 mud crabs out of 10 survived experimental trials in both the sand and shell hash habitat types. Reef habitats again tended to increase the survivorship of mud crabs in the presence of medium blue crabs, although not significantly. Small blue crabs did not prey on mud crabs in any habitat type, with on average 90% of mud crabs surviving (Fig. 2).Approximately 97% of all mud crabs survived in no-predator control trials (data not shown).

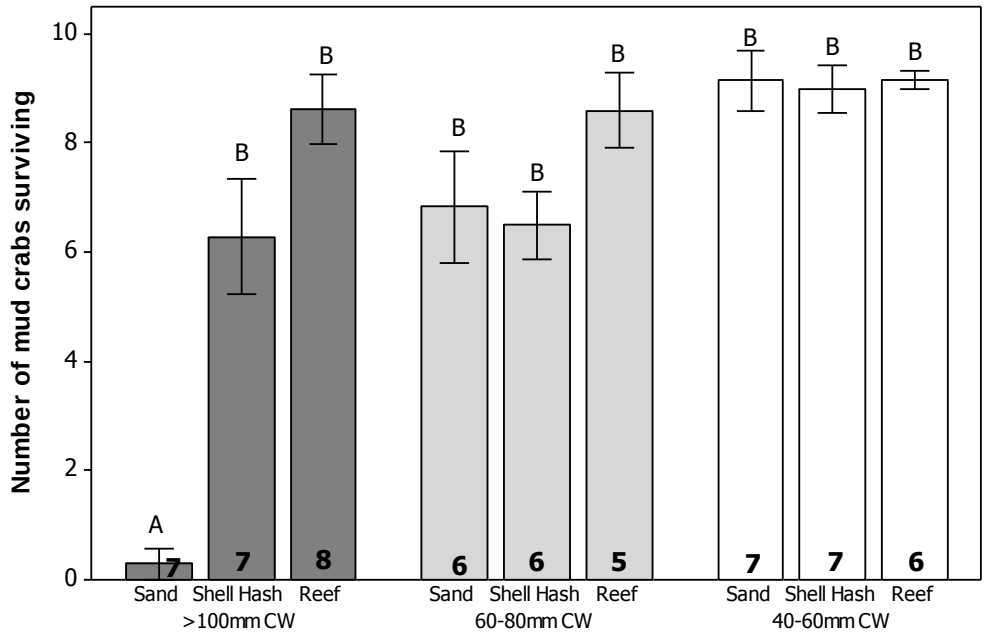

Figure 1.2. The effects of blue crab body size and habitat type on mud crab survivorship (mean ± SE) in lab mesocosms. Both habitat (ANOVA, $F_{2,50}$=18.73, P<0.001) and predator size (ANOVA, $F_{2,50}$=29.34, P<0.001) significantly affected mud crab survival but the magnitude was dependent upon the interaction between the two factor levels (ANOVA, $F_{4,50}$=13.22,P<0.001). Differing letters denote significant differences based on Tukey post hoc tests (P<0.05). The number of replicates is denoted by numbers at the base of graph bars. A total of 10 mud crabs were placed in each mesocosm.

Confirming oyster habitat refuge in the field

Increasing habitat complexity in natural environments significantly increased the survival of tethered mud crabs at each field site (Fig.3; PL; X^2, P=0.01; DMH; X^2, P=0.038). Only 40% of mud crabs were recovered in sand habitats at Priest's Landing, followed by 60% and 80% in shell hash and reef habitats respectively (Fig. 1.3a). Less than 40% of mud crabs were recovered from sand habitats at Dead Man's Hammock in contrast to 65% recovered from both shell hash and reef habitat types (Fig. 1.3b). Missing mud crabs were likely a result of predation as few mud crabs died or escaped their tethers in the lab. On average, 95% of all tethers were recovered from habitats at each site.

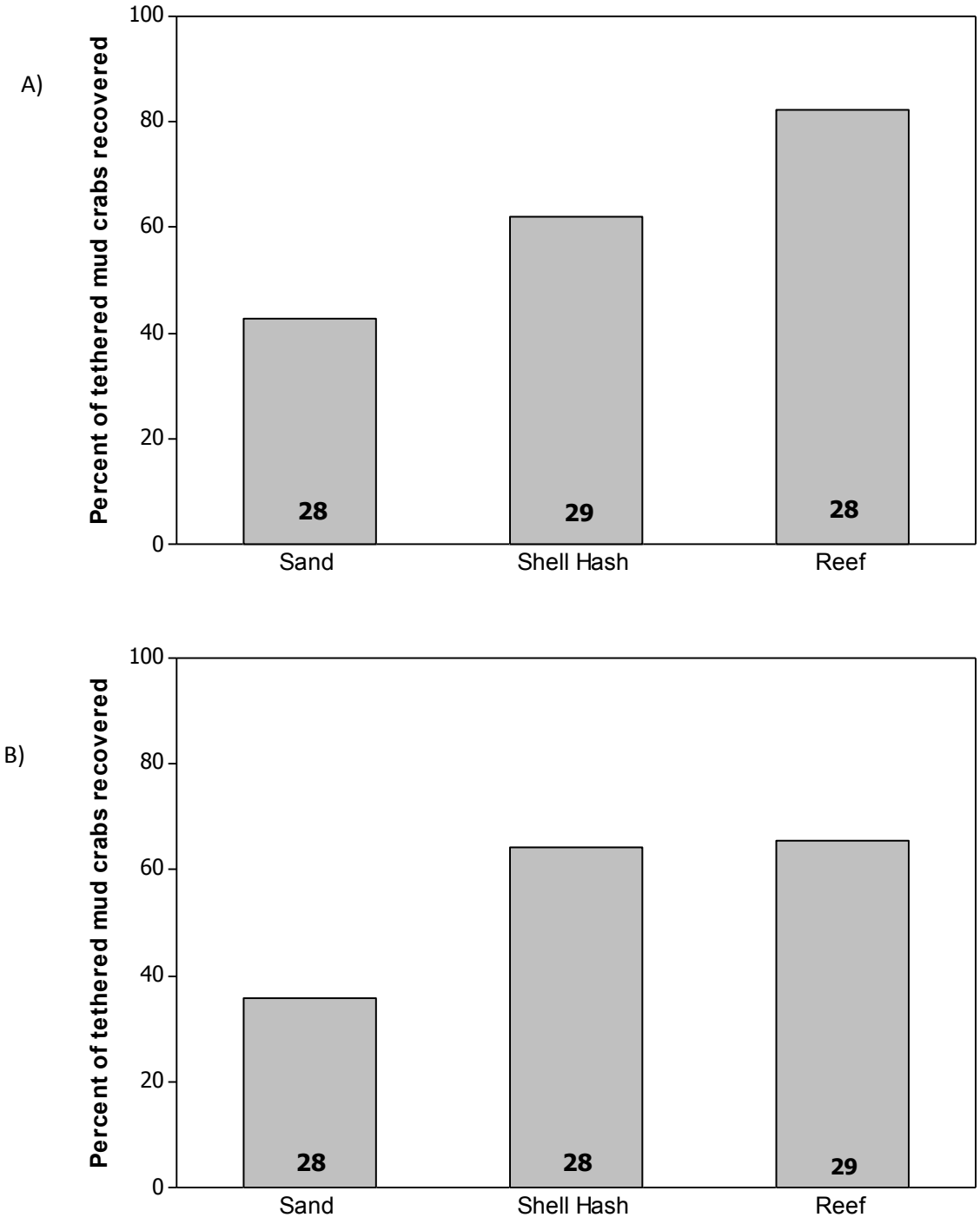

Figure 1.3. Percent of tethered mud crabs recovered in differing field habitats at A) Priest's Landing and B) Dead Man's Hammock. Increasing habitat complexity increased survival of tethered mud crabs at each site (PL; X^2, P=0.01; DMH; X^2, P=0.038). The total number of tethers recovered is denoted by numbers at base of graph bars.

Discussion

Ecological theory indicates that intraguild predation should be unstable and rare in natural systems (Polis et al. 1989, Polis and Holt 1992, Holt and Polis, 1997). However, blue crabs and mud crabs represent an intraguild predation system that is present in estuarine systems on both the Atlantic and Gulf coasts. My results suggest that habitat complexity and predator size may mediate interactions between these IG predators in which a) mud crabs are better consumers for oyster prey in complex habitats b) habitat complexity provided by oyster reefs offers mud crabs a refuge from predation and c) only large blue crabs are efficient predators on mud crabs.

Mud crabs consistently consumed more oysters in comparisons with blue crabs, which suggests that mud crabs are the dominant consumer of shared oyster prey (Fig. 1.1). On average, mud crabs consumed approximately 70% of oysters within laboratory oyster reefs. This result is expected as mud crabs are ideal predators on oyster reefs because their body size and chelae morphology allows them to penetrate spaces within oyster reef structure and crush attached oysters. However, it was unexpected that blue crab predation rates on oysters were so low, averaging only one oyster consumed over three days. The relatively low consumption of oysters by blue crabs in my experiment suggests that the 'guild' connection between these species is relatively weak. However, other species of bivalves, such as clams and mussels, are also consumed by both predators and may provide a greater overlap of consumption rates.

Previous experiments suggest that blue crabs are important predators on oysters (Eggleston 1990a, Eggleston 1990b) and are capable of consuming large numbers of oysters 15mm-35mm in shell height (Eggleston 1990). The difference in predation rates

between this and previous studies is likely due to differences in experimental design. Eggleston (1990) used oysters that were attached to loose oyster shells strewn through tanks, which allows greater access to oysters. In comparison, oysters in my experimental design were attached to vertical structure within oyster reefs. Thus, the structural complexity of reefs may preclude blue crabs with large chelae from accessing oysters in this experiment. The effects of realistic mimics of oyster reef structural complexity in this experiment suggests future studies should include adequate methods that replicate the effect of the habitat structural complexity in order to accurately assess predation rates.

Previous studies in IGP have documented that increased habitat complexity can promote the survivorship of IG prey. My results are consistent with this evidence as the structural complexity of oyster reefs provided refuge from blue crab predation in lab mesocosms Importantly, the value of habitat complexity as a refuge habitat was dependent upon predator size (Fig. 1.2). The significant interaction between predator size and habitat type likely occurred because smaller predator size classes of 60-80mm and 40-60mm CW were generally less efficient predators regardless of habitat type. Medium blue crabs (60-80mm CW) consumed only 3-4 mud crabs in sand and shell habitats and ~1 mud crab in oyster reef habitats. Thus, oyster reef habitat can benefit mud crabs in the presence of medium sized blue crab predators. Small blue crabs (40-60mm CW) consumed almost no mud crabs in any habitat type. This lack of predation was likely a result of the small predator's inability to crush and consume mud crabs and because behavior of mud crabs deterred small blue crab predators. When approached by a blue crab of any size, mud crabs used a classic meral spread display common to many decapods species (Dingle 1983) in which they outstretch their chelae. This behavioral tactic was ineffective in the

presence of large blue crabs (Hill, *personal observations*). However, small blue crabs were often deterred by the display and retreated from mud crabs (Hill, *personal observations*).

Whereas smaller blue crab predators consumed few mud crabs independent of habitat complexity, large blue crabs (>100mm CW) were significantly influenced by oyster reef structure. Increased habitat complexity increases the number of surviving mud crabs in laboratory mesocosms with large blue crabs (Fig. 1.2). Oyster reefs confer the greatest benefit with approximately 90% of mud crabs surviving in the presence of oyster reefs. This survivorship level is only slightly less than that of no predator controls at 97% demonstrating that oyster reefs provide an excellent refuge from large blue crab predation. Increased mud crab survivorship with increasing habitat complexity has been noted in response to toadfish predators (Grabowski 2004) suggesting that oyster reef provides a refuge for mud crabs from a variety of predators.

Although this and previous studies indicate that the structure associated with oyster reefs can mediate a variety of predator-prey interactions, many of these experiments were performed only in laboratory environments. The benefits of oyster reefs to mobile crab inhabitants have, to my knowledge, rarely been documented in field environments (but see Hollebone and Hay, 2008). Consequently, I confirmed the benefits of oyster reefs as a predation refuge in field tethering experiments where generally more mud crabs survived in habitats of increased habitat complexity. Mud crabs were recovered from oyster reef habitats at a greater frequency than shell hash and sand habitats at the Priest's Landing Site (Fig. 1.3a). The second field site, Dead Man's Hammock, showed a slightly differing pattern in that both shell hash and reef habitats resulted in similar rates of mud crab

survivorship (Fig. 1.3b). Oyster reefs may not have conferred a greater benefit at this site as a majority of reef clusters at this site are relatively small. Consequently, tethered mud crabs had only a modest area away from reef edges where they would be less vulnerable to predators. As I was unable to determine which predators are responsible for the consumption of tethered mud crabs, this difference could also have resulted with varying predator identities at each site. However, there is no *a priori* reason to believe that predator identities varied greatly between sites. Furthermore, increasing habitat complexity results in similar patterns of survivorship with other mud crab predators, such as stone crabs and red drum (Hill, *unpublished data*) suggesting differing predators are similarly affected by increased habitat complexity.

In their entirety, these results suggest that the intraguild predation system between blue crabs and mud crabs is mediated in estuarine environments based by resource use, oyster reef habitat refuges, and foraging efficiencies associated with blue crab predator body size. These interactions imply that blue crabs may have a positive indirect effect on oyster survival by controlling intermediate mud crab densities, especially if mud crabs are encountered outside highly structured habitats. Consequently, the combination of blue crabs and mud crabs may reduce predation risk in oyster prey. Although I did not test additive predator effects, Grabowski et al. (2008) supports this hypothesis by demonstrating that combinations of large blue crabs and mud crabs result in greater oyster survivorship than mud crabs alone. This risk reduction is believed to be a consequence of both blue crab consumption of mud crabs (a density effect) as well as antagonistic behavioral interactions between the two species (Grabowski et al. 2008). However, I have additional experiments demonstrating that mud crabs also modify their

behavior in the presence of large blue crabs resulting in increased oyster survival (Hill and Weissburg, Ch. 2). Thus, increased oyster survival in the presence of large blue crab predators is likely a result of a non- consumptive effect (Hill and Weissburg, Ch. 2) and not due to interference interactions. However, interference interactions between small blue crabs and mud crabs may also reduce the risk to oyster prey.

Behavioral modifications of IG prey in response to IG predators previously have been demonstrated between invasive crab predators in rocky intertidal systems where non-consumptive effects were responsible for over 80% of risk reduction to shared prey (Griffen and Byers 2006). Thus, behavioral modifications in response to predators are likely another important mechanism in mediating interactions of IG predators and prey. Furthermore, many studies often use additive predator experiments in differing habitats to examine emergent predator interactions. These studies are often demonstrating the effects of resource use, habitat structure, and animal behavior in emergent interactions (i.e risk reduction or risk enhancement) within intraguild predations systems. Although these studies are demonstrating factors which mediate IGP, antipredator behaviors have only recently been used in theory to facilitate the stability of IGP systems in ecological models (Nakazawa et al. 2010, Urbani and Ramos-Jiliberto 2010). I suggest that interactions of resource use, habitat complexity, and antipredator behavior are likely mediating IGP interactions across multiple systems. Future studies should examine emergent predator interactions in the context of intraguild theory in order to further demonstrate the mechanisms that allow intraguild predation to be abundant in nature.

In conclusion, both variances in habitat structure and predator body size mediate interactions between blue crab IG predators and mud crab IG prey. Mud crabs are the

primary consumer of oysters in this IGP system and successfully use oyster reefs as refuge from predation. Both the increased oyster consumption by mud crabs and their refuge from predation result from the increased habitat complexity of oyster reefs which preclude blue crabs from accessing both mud crabs and their shared oyster prey. These and other experiments (Grabowski et al. 2008) implicate blue crabs as a positive impact on oyster reef communities through top-down control of intermediate mud crab predators. These interactions may further be mediated by mud crab behavioral avoidance of blue crab prey providing a multitude of causes for the coexistence of blue crab and mud crabs across U.S. coastlines.

CHAPTER TWO

One crab, many crabs, small crab, large crab: Cue concentration, diet, and sensory context determine how prey perceive the risk associated with predator body size and density

Abstract

Predator body size is a significant predictor of predation risk in many aquatic communities. According to the threat sensitive predator avoidance hypothesis, prey should display antipredator responses that match the risk associated with predator body size. However, the ability of prey to assess predator body size via chemical cues is unclear. If prey can detect the risk associated with predator body size, *a priori*, this suggests that chemical cues associated with predator body size should propagate differing indirect non-consumptive effects. Yet, the importance of size-based non-consumptive effects and the context under which they occur is largely understudied. In this study, I investigated the role of predator body size and density in antipredator behavior, indirect interactions, and the mechanisms involved by examining the behavioral responses of mud crabs (*Panopeus herbstii*) and the survival of oyster prey (*Crassostrea virginica*) in response to various blue crab (*Callinectes sapidus*) predator treatments: one small blue crab, one large blue crab, multiple small blue crabs (i.e. increased concentration) and no predator controls. Mud crabs increased their refuge use and decreased their foraging in response to cues from large and multiple small blue crabs, but not single small blue crabs, demonstrating mud crabs perceive predator biomass via chemical cues. Mud crabs also responded to predator density as large vs. multiple small blue crabs caused differential antipredator responses if mud crabs perceived additional sensory information (i.e. auditory or visual). As blue crabs feed at differing trophic levels through their ontogeny,

mud crab responses to blue crabs varied depending on predator diet. These experiments demonstrate that we cannot successfully predict indirect interactions without considering predator population size structure and the contexts under which we determine predator risk.

Introduction

Predation is one of the most important forces in structuring ecological communities and imposes high selection pressure on prey to develop strategies to avoid being eaten (Dawkins and Krebs 1979). One such strategy is predator risk assessment in which prey use a variety of cues from multiple sensory modalities to determine predator threat levels, perform antipredator behaviors, and increase survivorship (Lima 1998). In aquatic environments, risk is typically assessed via chemical cues that transmit information such as predator identity, activity, and density (Kats and Dill 1998, Ferrari et al. 2010). Most importantly, antipredator behaviors dictated by predation risk can have large impacts on community structure due to their ability to propagate a wide variety of indirect interactions, known as non-consumptive effects (NCEs, Werner and Peacor 2003).

Due to the large impacts in community structure, current studies are focused on determining what information prey use to assess predator risk in order to predict outcomes for prey survival and NCEs. One of the greatest indicators of predation risk for prey is predator body size, which often determines the character and strength of many predation interactions by dictating habitat choice, diet width, prey size, and even consumption rates of predators (Werner and Gilliam 1984, Cohen et al. 1993). Further, these differing size-based predator traits lead to numerous cascading indirect interactions in communities (Dodson 1970, Werner and Gilliam 1984). However despite its

importance in predicting predator-prey outcomes, the role of predator body size in risk assessment and NCEs is still understudied. According to the threat sensitive predator avoidance hypothesis (Helfman 1989), prey will respond to predators with antipredator behaviors that match predator threat levels. Thus, if increasing predator size is indicative of greater threat, prey should display graded responses to differing predator size classes.

Currently, our knowledge about the chemical perception of predator size and the mechanisms by which it may occur is limited. In the context of chemical cues, predator body size may be perceived through differing cues or through chemical concentration associated with predator biomass. Although, evidence suggests that predator size is perceptible in chemical cues, the mechanism by which differential perception occurs is largely unknown. Two studies suggest that predator biomass is responsible for size-based risk perception. Pettersson et al. (2000) demonstrated that carp display avoidance responses to large pike but not small pike, but do not address if this is due to cue concentration. Additionally, Chivers et al. (2001) showed that sculpin responses to chemical cues from large predatory brook trout and multiple small brook trout (equal to concentration of large brook trout) were not significantly different, but did not examine responses to single small brook trout. In contrast, Kusch et al. (2004) exhibited that fathead minnows responded with more intense antipredator behavior to sympatric small pike than allopatric large pike predators suggesting that differential perception is a result of differing cues. Most importantly, these studies do not demonstrate whether size-based risk translates into differences for prey survival or cascading indirect interactions. This is due to common methodological practices in which assessing predator risk often involves measuring only prey behavior in response to visual or chemical cues to predators but not

prey survival or indirect interactions. Thus, the ability of predator size to propagate indirect interactions through differential risk assessment is still unknown.

However, predator size is not the only context along which prey assessment of predator risk can vary. Predator diet often affects a prey's evaluation of risk whereby diets including conspecifics often result in the greatest antipredator responses (Schoeppner and Relyea 2005, Turner et al. 2008, Ferrari et al. 2010). Other diets can induce antipredator responses, but these responses often decrease with increasing phylogenetic distance of the diet (Schoeppner and Relyea 2005). Additionally, in systems where predator body size dictates the trophic level where predators feed (i.e. intraguild predation systems), diet cues may be beneficial to determining the threat of oncoming predators. Consequently, diet cues are likely utilized to further determine predation threat beyond predator size.

In addition to diet, the sensory landscape, or the variety of cues (chemical, visual, auditory etc) available to prey, also affect antipredator behavior. For instance, Ward and Mehner (2010) demonstrated that mosquitofish displayed differing level antipredator responses to visual and chemical predator cues. Fish predators also show differential refuge habitat use in response to differing sensory cues (Martin et al. 2010). Additionally, abiotic factors, such as turbidity and turbulence, can interfere with prey perception of predators (Hartman and Abrahams 2000, Smee et al. 2008, Ferrari et al. 2010b). The inability of prey to detect predators can result in the loss of antipredator behaviors which propagate NCEs (Smee et al. 2008). Unfortunately, many studies, particularly those examining anti-predator behaviors generally, and NCEs specifically, use experimental paradigms that make it difficult to establish the importance of multiple sensory cues. For instance, the use of predator metabolites or body washes is extremely common (Kats and

Dill, 1998), but may mask the importance of visual or other cues that may be combined with information gleaned from predator scent.

As multiple contexts including predator size may affect predator risk assessments, I had multiple objectives in my study. I wanted to: a) identify the role of chemical cues in assessing size-based predation risk; b) investigate the role of predator biomass in differential perception c) determine whether antipredator behaviors based on predator biomass cause differing magnitude non-consumptive effects; d) examine whether diet may play a role in the risk response and; e) identify differences in sensory context that can affect antipredator behaviors and interfere with the predictions of NCEs.

Methods

Model System

In order to examine the role of predator biomass, sensory context, and diet in predator risk assessment and NCEs, I chose an intraguild predation system consisting of both adult and juvenile blue crabs (*Callinectes sapidus*), mud crabs (*Panopeus herbstii*), and their shared oyster prey (*Crassostrea virginica*). This system is ideal for examining size-based interactions because blue crabs are generalist predators (i.e. diet varies), predation by crabs is crush-limited, prey size scales with predator body size, and because size classes co-occur. The IG predator in this system, blue crabs (*Callinectes sapidus*), are important predators and scavengers of estuarine environments (Micheli 1997) and have been shown to prey on a variety of bivalve and crustacean species (Eggleston 1990a, Eggleston 1990b, Fitz and Weigert 1991, Micheli, 1997). The IG prey, mud crabs (*Panopeus herbstii*), are small cryptic xanthid crab predators that are found in both oyster reef and salt marsh habitats. Mud crabs occupy the interstices of oyster beds at high

densities (Lee and Kneib 1994, Hollebone and Hay 2007) and prey on a number of bivalve species (Seed 1980, Bisker and Castagna 1987). Xanthid and other non-portunid crabs make up approximately 43% of the diet of blue crab (Fitz and Weigert 1991). Furthermore, mud crab risk to predation varies as a function of blue crab predator size; large adult blue crabs (>100mm carapace width; CW) are voracious predators on mud crabs in lab mesocosms, whereas small juvenile blue crabs (40-60mm CW) rarely present a threat to mud crabs greater than 15mm CW (Hill and Weissburg, Ch. 1). Thus, according to the threat sensitive predator avoidance hypothesis (Helfman 1989), mud crabs should respond to larger blue crab body sizes with increased magnitude antipredator behaviors. *A priori*, this also suggests blue crab body size may propagate differing levels of NCEs.

Animal collection and maintenance

All experiments were performed at the Skidaway Institute of Oceanography (SkIO), Skidaway Island, Georgia, USA over summer months in multiple years from 2008-2010. Both blue crabs and mud crabs were collected from Wassaw Sound and associated tributaries. Blue crabs were collected by commercial crab pot and seine net. Mud crabs were collected by hand from loose oyster reef. Hatchery-reared oysters (10-15mm in length) were obtained from Bay Shellfish (Tampa, FL). All animals were maintained in covered outdoor flow-through seawater tanks at the SkIO for a minimum of 48 hours before experiments began. Blue crabs were maintained on a diet of shrimp and/or clams and were fed an *ad libitum* diet of shrimp and oysters once a day for 48 hours prior to experiments. Mud crabs were maintained on a clam diet and were starved 48 hours prior to experiments.

Investigating the role of predator biomass

In order to examine the ability of prey to distinguish predator body size and density via chemical cues, I monitored mud crab foraging on oysters in response to differing biomasses of caged blue crab predators in laboratory mesocosms (Summer 2008). Mesocosms (0.7m x 0.4m x0.3m) consisted of artificial oyster reefs constructed over approximately 2.5 cm of sand and shell hash substrate supplied with flow-through seawater. Artificial reefs were constructed by gluing 10 oyster shells (obtained from natural shell banks) to create similar small clusters (approximately 6 cm in diameter), and then bundling clusters with rubber bands to build a reef (21 clusters per tank). One juvenile oyster (10-15mm in length) was secured on the face of each cluster using cyanoacrylate glue (21 oysters total). By using artificial reef structure, I could control the placement of juvenile oysters within reefs; natural heterogeneous clusters prevented minimizing variation between tanks and also altered the ability of mud crabs to access oyster prey. Once the reef structure was created, approximately 1 liter of shell hash was scattered around reef edges to mimic the natural structure of the habitat and to help hold the reef in place. Twenty mud crabs (similar to natural size class densities- twelve 15-20mm; four 20-25mm; three 25-30mm CW; Lee and Kneib, 1994) were then added to tank. Each mud crab received a painted fluorescent mark on its carapace in order to monitor its behavior with a black light wand at night when mud crabs are typically more active (Grabowski 2004). One of four caged predator treatments was submerged into the tank once mud crabs were added; one large blue crab (>100mm CW; approximately 130-180g), one small blue crab (40-60mm CW; 8-20g), multiple small blue crabs (40-60mm CW; totaling 130-180g), and a no predator control. Multiple small blue crab treatments

matched the weight of large blue crabs and were used to address the role of cue concentration via biomass in predator risk assessment. Predator cages consisted of translucent plastic containers (0.34m x 0.20m x 0.12m) with multiple holes drilled through the side and a vexar mesh cover in order to allow movement of chemical cues into mesocosms, but prevented blue crabs from having any direct contact with mud crabs. Blue crabs were fed an *ad libitum* diet of shucked oysters every day as this diet is common to multiple sizes of blue crab predators (Eggleston, 1990b). Mud crabs were allowed to forage on oysters in mesocosms for 2.5 days. The total number of oysters eaten was recorded at the conclusion of the experiment, as well as the number of surviving mud crabs. At night, the activity of mud crabs in mesocosm reefs was monitored using a black light wand to illuminate the fluorescent paint on mud crab carapaces (Grabowski, 2004). The number of fluorescing mud crabs visible in tanks was counted once every 15 minutes for 30 minutes each night of the experiment (9 observations total). Mud crabs that were not visible were either buried underneath sand or hidden within oyster reef structure. I calculated the average percentage of mud crabs visible on reefs by dividing the average number of mud crabs visible over three nights by the total number of mud crabs surviving experiments. Due to limited mesocosm space, three replicates were run at one time in a randomized block design, with six runs in all, totaling eighteen replicates for each treatment. Run(time) was used as a factor in analysis as preliminary analysis demonstrated that run(time) significantly affected the behavior and percent of oysters eaten. Thus, the average percentage of mud crabs visible was analyzed by two-factor ANOVA for run and predator biomass treatment. The percent of oysters eaten was arcsine transformed to meet assumptions of normality and analyzed by

a two factor ANOVA for the effects of run and predator treatment. The number of mud crabs surviving also was analyzed via a two-factor ANOVA (run, predator size) in order to determine if significant differences in mud crab survival over time or by treatment may have significantly impacted oyster survival.

Chemical vs. Multiple Sensory Cues

In the previous experiment, observations of large and multiple small blue crabs within predator boxes revealed there were multiple sensory stimuli that may have allowed mud crabs to differentiate large and multiple small blue crab predators. Large blue crabs were generally quiescent in predator boxes and moved very little even during feeding. In contrast, multiple small blue crabs often fought for both space and food within boxes and consequently moved constantly creating additional sounds and shadows not seen in large blue crab treatments. Thus, I performed an additional experiment to examine mud crab responses to multiple small and large blue crabs with only chemical cues present (Summer 2010). Isolating chemical cues from other sensory stimuli was accomplished by separating predator boxes from foraging mud crabs by suspending boxes above mesocosms. This allowed seawater to flow into predator boxes and then drain into mesocosms containing foraging mud crabs with no other sensory stimuli. Predator boxes were similar to previous experiments except only five holes (~2mm diameter) were drilled into the bottom of plastic boxes to allow seawater to fill predator boxes and drain along with chemical cues through the bottom. The bottoms of these containers were also painted black to ensure that movement or shadows could not be observed by mud crabs in mesocosms. Mesocosms were set up as in previous experiment containing artificial oyster reef, 42 juvenile oysters (two 10-15mm oysters per cluster), and twenty mud crabs. Blue

crabs were fed an *ad libitum* diet of shucked oysters every day. The number of oysters eaten was counted every day for 2.5 days. Observations of mud crab presence on reefs at night was also recorded as in previous experiment. Five replicates of multiple small and large blue crabs were run at a time over two runs for a total of 10 replicates. As preliminary analysis established run (time) had no significant affect on the percent of oysters eaten, all data from runs were pooled. The percentage of oysters eaten as well as the average percentage of mud crabs visible on reefs at night was analyzed by ANOVA for the effects of predator treatment. The percent of mud crabs visible was analyzed by a two factor ANOVA (run, predator treatment) as run (time) significantly affected mud crab activity.

Predator diet

In order to examine the effect of blue crab diet on the level of mud crab antipredator response, I investigated mud crab foraging behavior and oyster survival in response to a caged large blue crab fed one of two diets, shucked oysters or mud crabs, in comparison with crushed conspecifics (mud crabs), and zero predator controls (Summer 2009). Since previous studies have demonstrated crushed conspecific cues alone can induce antipredator activity (Ferrari et al, 2010), crushed mud crab treatments were used to determine whether increased responses to blue crabs could be attributable to alarm cues from crushed conspecifics alone or were additive with blue crab cues. Mesocosms were setup as in previous mesocosm experiments containing artificial oyster reef, 42 oysters (two 10-15mm oysters per artificial cluster), and twenty mud crabs. Blue crab diet treatments consisted of large blue crabs caged in predator boxes (as in predator size experiments) and placed in mesocosms. Blue crabs were fed on an *ad libitum* diet of

shucked oysters or crushed mud crabs once a day. Crushed mud crab treatments were applied once a day at the same time as blue crab feeding events and consisted of one large (25-30mm CW), two medium (20-25mm CW), and two small (15-20mm CW) crushed mud crabs. Multiple sizes of mud crabs were used as differing sizes of conspecifics may elicit different reactions by prey (Mirza and Chivers 2002). Crushed mud crab treatments were prepared by quickly culling crabs with a mallet in 0.7 liters of seawater and then cutting them in half with scissors to mimic a blue crab predation event. Crushed conspecifics were then added immediately to a predator box within the mesocosm. Remnants of crushed mud crabs were removed directly before the next application of crushed conspecifics cue. The number of oysters eaten was recorded every 24 hours for 48 hours. The presence of mud crabs on reefs was also monitored at night as in previous experiments. Due to limited mesocosm space, three replicates were run at a time for four runs totaling twelve replicates per treatment. As predation rates were high during the experiment, I analyzed the percent of oysters eaten after 24 hours, which were square root transformed to meet assumptions of normality, by a two-factor ANOVA (run, diet) since preliminary analysis indicated that run (time) affected the percent of oysters eaten. The average percent of mud crabs visible at night over the course of the experiment analyzed by two-factor ANOVA (Run, Predator Diet).

Results

Predator biomass

Chemical cues from blue crab predators had a significant effect on the percentage of oysters eaten by mud crabs (Fig. 2.1a; $F_{3,48}$=7.75, P<0.001). Experimental run (time) also had an effect on the number of oysters eaten ($F_{5,48}$=5.41, P<0.002) but there was no

interaction between run and predator treatment ($F_{15,48}$=1.62, P > 0.l0). Cues from both large and multiple small caged blue crabs suppressed the percent of oysters eaten by mud crabs by approximately 20-25%. In contrast, single small blue crabs did not suppress mud crab foraging, which was not significantly different from zero crab controls (Fig. 2.1a). The number of mud crabs surviving the duration of the experiment was significantly affected by run (N=18, $F_{5,48}$=3.02, P <0.02), but not by predator treatment ($F_{3,48}$=0.73, P >0.50). The same results for ANOVA and post-hoc tests were obtained when the number of oysters eaten in each tank was corrected for differences in mud crab survival by dividing the predation by the number of surviving mud crabs.

The presence of mud crabs on mesocosm reefs during nighttime observations was significantly affected by blue crab predator size (Fig. 2.1b; $F_{3,48}$=8.83, P<0.001) and run ($F_{5,48}$=4.74, P<0.002), but there was no significant interaction (Treatment × Run, $F_{15,48}$=1.59). Large blue crabs suppressed the percent of mud crabs visible on reefs by approximately 10% indicating that large blue crabs caused mud crabs to seek refuge deeper within reefs where they could not be observed (Fig 2.1b; Grabowski 2004). The presence of mud crabs was not suppressed in response to either small or multiple small blue crabs. The lack of response to multiple small blue crabs was unexpected as multiple small blue crabs caused an increase in oyster survival indicating that multiple small crabs may have induced antipredator behavior. This suggests that mud crabs were not foraging on oysters in response to multiple small blue crabs, but this increase in survival could not be attributed to mud crabs seeking refuge deeper within reefs as might be expected for prey responding to predator threat.

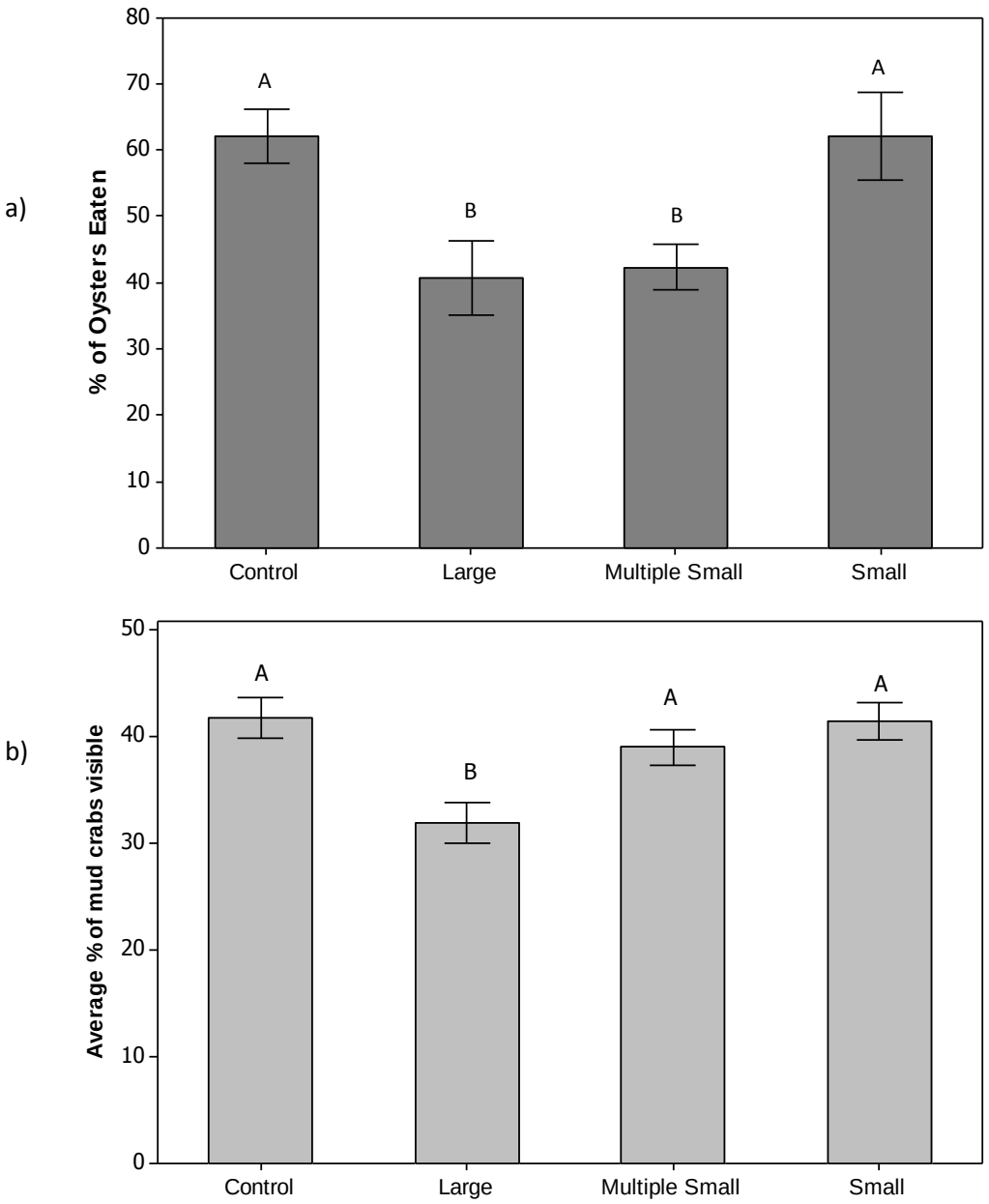

Figure 2.1. Percent of a) oysters eaten (mean ± SE) b) mud crabs visible outside of reef refuges (mean ± SE) in response to blue crab biomass treatments. Differing letters denote significant differences based on Tukey post-hoc test ($P<0.05$).

Chemical vs. Multiple Sensory Cues

Confining blue crab predators in predator boxes in the previous experiment exposed prey in these treatments to several sensory stimuli (i.e. shadows, movement, noise) in addition to chemical cues. The presence of additional auditory or visual stimuli may act in conjunction with odor to either detect predators and/or determine predator size. This prevented us from being able to conclude that prey responses to the multiple small blue crab reflected the role of chemical cue concentration in mud crab perception of predator size. Thus, it was necessary to compare large and multiple small blue crabs exposed only to effluent containing chemical cues from the blue crab predators.

In agreement with the predator size experiments, multiple small and large blue crabs caused similar levels of predation on oysters (Fig. 2.2a; $F_{1,18}$=0.05, P=0.822). However, contrary to the previous experiment, presenting only chemical cues from predators caused mud crabs to behave similarly to large and multiple small blue crabs (Fig. 2.2b; $F_{1,16}$=1.03, P=0.326). Approximately 35% of mud crabs were visible in response to large and multiple small blue crabs. This indicates that mud crabs perceive the same threat from large and multiple small blue crabs when presented with only chemical cues. Run (time) affected the percent of mud crabs visible ($F_{1,16}$=11.22, P<0.005), but there was no run×treatment interaction ($F_{1,16}$=2.31, P=0.148)

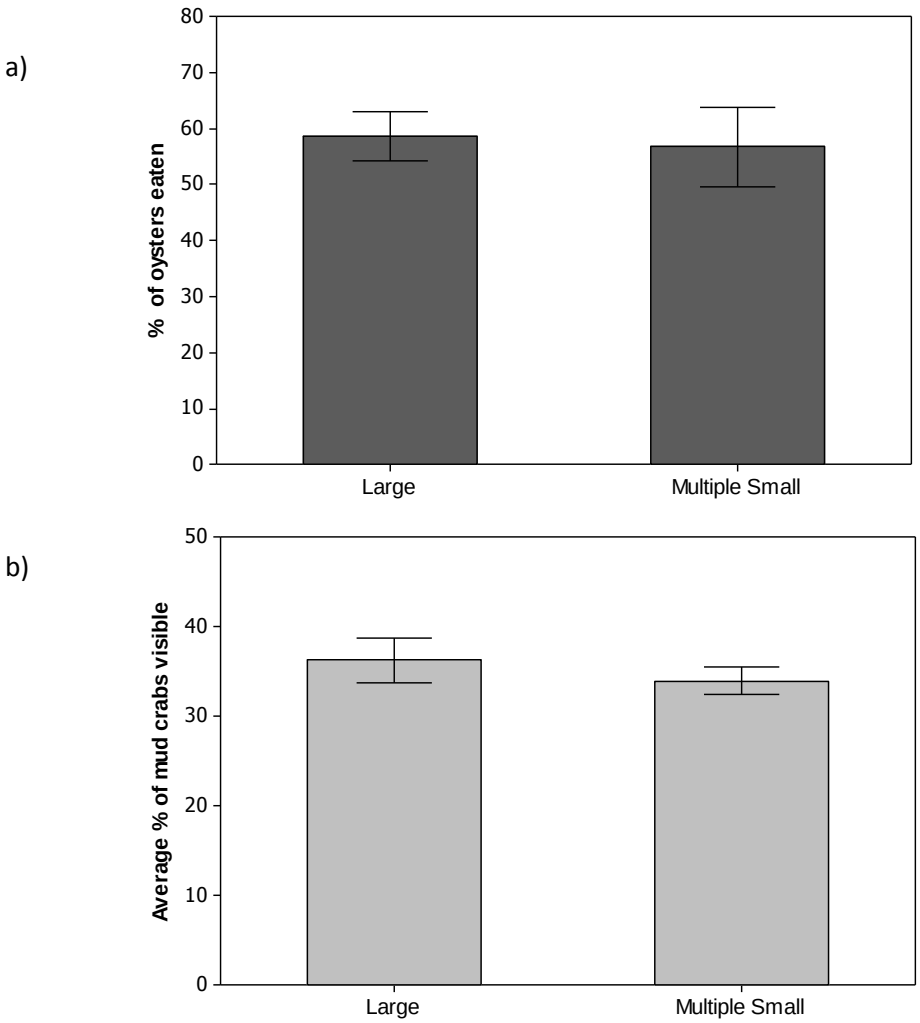

Figure 2.2. The percent of a) oysters eaten (mean ± SE) and b) mud crabs visible outside of reef refuges (mean ± SE) in response to isolated chemical cues from large and multiple small blue crabs. When exposed to chemical cues alone, mud crab responses to large and multiple small blue crabs were not significantly different (% eaten, $F_{1,16}$=0.05, P=0.822; % mud crabs visible, $F_{1,16}$=1.03, P=0.326)

Predator Diet

Blue crab diet ($F_{3,32}$=4.85, P=0.007) and experimental run ($F_{3,32}$=3.89, P=0.018) both had a significant effect on the number of oysters eaten (Fig. 2.3a). The run × treatment interaction was not significant ($F_{9,32}$=0.40, P=0.925). Both blue crabs fed oysters and crushed mud crabs alone suppressed the percentage of oysters eaten by approximately

15% from controls but were not significantly different. Blue crabs fed mud crabs decreased predation on oysters the greatest amount, on average 30% (Fig. 2.3a). The average percent of mud crabs visible outside of reef refuges was significantly affected by run ($F_{3,32}$=5.01, P=0.006) and diet treatment ($F_{3,32}$=11.57, P<0.001) and closely followed the patterns of predation (Fig. 2.3b). Blue crabs fed oysters and crushed mud crabs both suppressed the number of mud crabs visible by ~10-12% (Fig. 2.3b). The greatest suppression of mud crabs outside of reefs was ~20% and caused by blue crabs fed mud crabs. The treatment × run interaction was not significant ($F_{9,32}$=0.66, P=0.737).

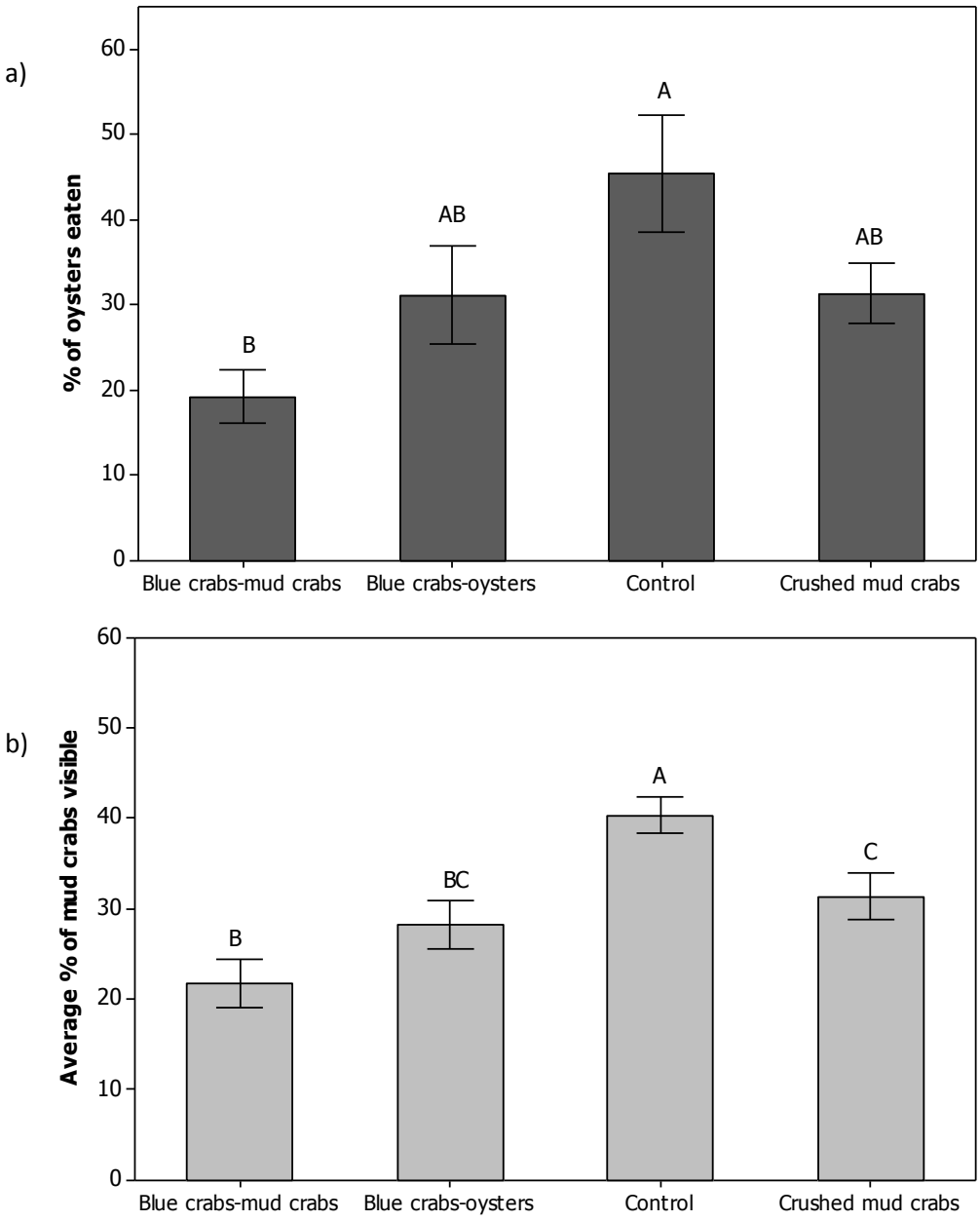

Figure 2.3. Percent of a) oysters eaten (mean ± SE) and b) mud crabs visible outside of reef refuges (mean ± SE). Blue crab-mud crabs denotes blue crabs fed mud crab diet. Blue crabs-oysters denotes blue crabs fed oyster diet. Differing letters denote significant differences based on Tukey post-hoc test ($P<0.05$).

Discussion

These results add to a body of evidence that indicates prey use a variety of information from multiple sensory sources in order to assess their predation risk from predators. In my study, blue crab body size, density, diet, and sensory context all impacted the antipredator behavior of foraging mud crabs and altered the magnitude of NCEs on oyster prey. Blue crab body size was a significant predictor of the magnitude of NCEs as mud crabs differed in their behavioral responses to chemical cues from individual small and large blue crabs. Large blue crabs suppressed the activity of mud crabs during nighttime observations and the percentage of oysters eaten on oyster reefs similar to mud crab behaviors observed in other studies (Fig. 2.1; Grabowski 2004, Grabowski and Kimbro 2005) Thus, large blue crabs are perceived as risky resulting in an indirect NCE that increases oyster survival. In contrast, a small blue crab did not cause any measured response from mud crabs in either behavior or oyster predation. Previous predation experiments between mud crabs and blue crabs documented that small blue crabs do not prey on mud crabs, whereas large blue crabs consume mud crabs at high rates (Hill and Weissburg Ch. 1). Thus, the differential response to individual large and small predators is consistent with the threat sensitive predator avoidance hypothesis (Helfman, 1989) which predicts that prey will display risk appropriate responses to differing predator threat. These size-based risk assessments then cascade to the next resource level causing differing magnitude NCEs. To my knowledge, this is the first demonstration of how differing predator body sizes cause differences in risk perception and change the magnitude of indirect NCEs.

Differential perception of large and small size classes is based on both chemical and non-chemical cues. This conclusion reflects observations from two different experiments that exposed mud crabs to different cues from large and small blue crab predators. Multiple small blue crabs in submerged predator boxes provided mud crabs with additional sounds and possibly shadows and movement not perceived in individual crab treatments. In response to additional sensory information, mud crab activity outside of reefs was significantly different in response to large and multiple small predators (Fig. 2.1b). However, when predator boxes were mounted outside of mesocosms so that only chemical cues were reaching mud crabs, responses to multiple small and large blue crabs in either predation or refuge use were not significantly different (Fig. 2.2a,b). Consequently, when limited to chemical cues, mud crabs cannot differentiate large predators from high densities of small predators as responses are associated with cue concentration and biomass. It is unclear whether this perception of risk would occur under natural conditions where odors move via plumes that vary in concentration (Weissburg 2000) and small blue crabs would be distributed over a larger area (i.e. cues are more dispersed). This likely would depend on the environmental conditions, spatial distributions of predators and prey, and the encounter rate with small predators. Smaller predators that have a high density, are spatially aggregated, or have a high encounter rate with prey may be more likely to induce risk responses than small predators at lower densities.

As demonstrated by my experiments, the sensory environment plays a role in whether mud crabs can perceive density associated with predator biomass. Thus, the determination of large vs. multiple small predators in natural environments may be

dependent upon the ability of prey to interpret a variety of visual, auditory, and chemical cues. Other previous studies have also demonstrated that sensory context can influence the perception of risk in vertebrate species. For instance, Chivers et al. (2001) showed that slimy sculpin differentially respond to many small vs. large predators on the basis of visual but not chemical cues. In addition, in Martin et al (2010), juvenile roach (*Rutilus rutilus*) differed in their refuge use if they were exposed to only olfactory or visual cues from fish predators; roach preferred structured habitat if they smelled pike predators but remained in open habitat in response to only visual cues. Yet, the role of multiple sensory signals in NCEs is relatively understudied in comparison to single modalities (but see Hartman and Abrahams 2000, Chivers et al. 2001, Ward and Mehner 2010). The sensory complementation hypothesis (Lima and Steury 2005) predicts that multiple cues should function additively or synergistically in predator detection. This suggests that by only examining one sensory modality in antipredator behavior we may be misestimating NCEs due to a sensory deficit in empirical studies.

Interestingly, due to additional sensory information, the behavior (i.e. refuge use) of mud crabs was not predictive of risks associated with the concentration of predator cues, nor predictive of the impacts on the next resource level (i.e. both predation and activity outside refuges were not suppressed). This is important as behavioral ecologists frequently perform experiments where only prey activity level or refuge use is quantified to measure and interpret risk assessment by prey; increased risk often induces larger suppression of activity or different patterns of activity. In turn, changes in activity are assumed to directly reflect potential effects on downstream organisms. However, as my examinations demonstrate, measuring activity or refuge use may not be sufficient to

predict the level of risk that prey perceive or accurately interpret cascading interactions. This may be due in part to less than holistic tests of behavior that may not accurately interpret prey decisions. In order to fully understand both prey responses, as well as its impacts on community structure, future studies in predator risk assessment should measure multiple interactions such as prey survival and outcomes to other resource levels.

Dietary cues also played a role in mud crab evaluations of blue crab predation risk. Mud crabs responded to blue crabs fed crushed conspecifics with greater intensity than blue crabs fed oysters (Fig. 2.3) demonstrating the consumed conspecifics represent a greater threat. Consistent with previous studies of predator diet, the response of predators fed conspecifics was even stronger than crushed conspecifics alone suggesting that the addition of alarm cues from crushed conspecifics was not enough to induce intense antipredator responses (Schoeppner and Relyea 2005; Schoeppner and Relyea, 2009). Although many previous studies have identified that diet cues affect predator risk (Ferrari et al. 2010), the response of prey to predator diet may be particularly apt in an intraguild predation system where consumers may be either competitors or predators. Dietary cues may be beneficial predictors of risk in these systems because they indicate the trophic level where predators are feeding. Although my study supports this hypothesis, other studies in intraguild predation systems have not come to the same conclusion. Magalhaes et al. (2004) determined that intraguild prey responded only to predators fed shared prey but not conspecifics. In contrast, Choh et al. (2010) concluded that although intraguild prey respond to predator presence, the antipredator response does not vary with diet. At this point, I can only speculate as to reason these responses vary; however, it is possible

that responses to predator diet are based on the way that prey learn to associate cues with predators (Magalhaes et al. 2004). For instance, prey often learn to associate predators with risk after being exposed to combinations of predator odor and injured conspecifics (Mirza and Chivers 2001). Future studies should continue to examine the role of diet in predator risk assessment in order to determine circumstances that establish prey responses to diet cues.

In conclusion, predator body size, density, and the diet of blue crabs modulate the level of antipredator behavior of mud crabs and cascading non-consumptive effects. The perception of risk associated with predator size is mediated by the concentration or flux of chemical cues produced by differing biomasses of predators. Sensory context also affects the magnitude of antipredator behavior displayed by prey and can impact whether antipredator behaviors are predictive of cascading interactions. Similar recent studies support the idea that sensory modality by which we assess predator risk will impact the magnitude and direction of antipredator behavior. Future studies in predator risk assessment and NCEs should explore the role of both predator biomass and sensory context in order to accurately predict the role of NCEs in communities. This will likely involve measuring prey survival, and/or indirect interactions in order to display predator risk in the context of natural communities.

CHAPTER THREE

Predator biomass determines the magnitude of non-consumptive effects (NCEs) in both laboratory and field environments

Abstract

Predator body size often indicates predation risk, but its significance in non-consumptive effects (NCEs) and predator risk assessment has been largely unstudied. Here, I evaluated the role of predator body size and biomass on risk assessment and the magnitude of NCEs by investigating mud crab foraging behavior and oyster survival in response to differing biomasses of blue crab predators using both laboratory and field methods. Cues from high predator biomass treatments including large blue crab predators and multiple small blue crab predators decreased mud crab foraging and increased oyster survival, whereas mud crab foraging in response to a single small blue crab did not differ from controls. Mud crabs also increased refuge use in the presence of large and multiple small, but not single small blue crab predators. Thus, both predator biomass and aggregation patterns may affect the expression of NCEs. In the field, flow characteristics were monitored over time and within cages and confirmed that flow conditions were representative of natural environments. Differences in flow over time were not associated with changes in NCEs. This study suggests that estimating NCEs without understanding the impact of predator biomass will not successfully predict the role of NCEs in shaping community dynamics.

Introduction

Predator–prey interactions, such as those responsible for trophic cascades, were originally thought to be purely consumptive interactions whereby predators reduced densities of

prey through lethal interactions and caused cascading indirect effects. However, increased attention has been focused on the non-lethal effects of predator intimidation and fear, dubbed non-consumptive effects (NCEs), in which prey actively change traits such as behavior, morphology, and habitat use in response to the presence of a predator (Lima 1998; Werner and Peacor 2003). In many cases, these changes are mediated by chemical cues emanating from predators or injured conspecifics that warn prey of imminent risk (Kats and Dill 1998). NCEs can account for up to 85% of the effects seen in trophic interactions based on meta-analysis of studies comparing NCEs with their traditional consumptive counterparts (Preisser et al. 2005). The importance of NCEs has prompted a number of studies into behavioral interactions and even revisitation of classic ecological predator-prey paradigms in order to investigate the role of NCEs in trophic dynamics (Peckarsky et al. 2008).

Much like consumptive effects in traditional trophic cascades, investigations into NCEs find that the magnitude and direction of their effects are largely context-dependent based on a number of factors including habitat type and complexity (Trussel et al. 2006; Ferner et al. 2009), resource levels (Bolnick and Preisser 2005), and predator identity (Turner et al. 1999; Schmitz 2008). This context dependence is driven largely by the evolutionary, sensory, and behavioral ecology of the prey that determine the set of conditions to which prey respond and their antipredator strategies (Kats and Dill 1998; Schmitz et al. 2004). Antipredator strategies frequently are dictated by the risk that prey assess using a variety of information about predators, such as type and activity (Stankowich and Blumstein 2005; Ferrari et al. 2010). Much of this information is perceived through chemical cues that mediate predator-specific behavioral responses in many prey species (Turner et al.

1999; Schmitz, 2008). Here, risk assessment based on the intensity and composition of chemical cues causes different magnitudes of antipredator behavior. For instance, tadpoles respond to increasing cue concentration and to phylogenetic relatedness of predator diet causing varying levels of both behavioral and morphological responses (Van Buskirk and Arioli 2002; Schoeppner and Relyea 2005). These differential antipredator strategies based on risk assessment can then lead to context-dependent NCEs on resources and ecosystem dynamics (e.g. Schmitz 2008). Consequently, it is necessary to account for predator traits that lead to differences in predator risk assessment in order to predict the magnitude of NCEs across ecosystems.

One aspect of predator variation that has been under-investigated in NCEs is the role of predator body size. Predation risk often is dictated by predator body size (Werner and Gilliam 1984; Cohen et al. 1993), either as a result of size-dependent predation mechanics (i.e. gape limited or crush limited predation) or because predator body size often determines habitat choice, prey choice, and even consumption rates of organisms (Werner and Gilliam 1984). Thus, predator body size affects the character and strength of many predator-prey interactions, both direct and indirect, resulting in differing community structure (Dodson 1970; Werner and Gilliam 1984; Rudolf 2006). As with consumptive effects, examinations of NCEs also must consider potential size-dependent predator effects.

Although current methods of characterizing NCEs have been critical for establishing the importance of behavioral interactions, the effects of predator body size in NCEs, or the predator characteristics that mediate potential size-dependent responses are relatively under-investigated. For instance, studies of chemically-mediated NCEs often involve

measuring the responses of prey to chemical cues from only a single (generally large) predator size class. Such methods may not account for potential size-specific interactions that can occur if prey can accurately judge, and respond to, the risk associated with predator size. In the context of chemical perception, the distinction between large and small predators could be made based on either qualitatively differing cues from each size class or via concentration as a function of predator biomass. The latter suggests that the combination of both predator size and degree of predator aggregation (i.e. biomass) will be an important factor in determining the magnitude of NCEs.

Previous studies have examined the role of predator biomass in modulating prey traits by constructing dosage-response curves using differing predator abundances (Van Buskirk and Arioli 2002; Schoeppner and Relyea 2008). However, rarely have studies determined the role of predator biomass vs. individual size in either influencing predator risk assessment (but see Chivers et al. 2001) and the propagation of NCEs to trophic levels other than that of the focal prey. Further, those aquatic studies that have examined the role of predator abundance in modulating prey behavior have done so in laboratory mesocosms where chemical cues from multiple predators can concentrate. In the natural environment, chemical cues move as odor plumes where cue concentration is often patchy and unpredictable (Weissburg 2000) suggesting that assessing predator biomass via chemical concentration in natural environments may be problematic.

Studies in simplified laboratory or mesocosm environments have been important in validating that predators can produce effects solely by changing prey behavior. However, the relationship of these studies to patterns of NCE strength in natural environments is unclear since they have been performed in simplified environments that do not reproduce

patterns of natural water flow that may alter the perception of predator chemical cues necessary for producing anti-predator behaviors (Weissburg 2000; Powers and Kittenger 2002; Smee et al. 2008; Ferner et al. 2009). In natural flow environments, NCEs may be more likely to be expressed in environments with low velocity or mixing conditions where predator cues may be easily detected (Smee et al. 2008). These conditions may change over time and may be highly dependent on local conditions (Smee et al. 2010) suggesting that monitoring environmental conditions may be necessary to explain variation in NCEs. Thus, performing NCEs in the field and characterizing the environment where NCEs occur can provide insights into the importance and context-sensitivity of NCEs in nature.

I examined the ability of prey to judge size and biomass-based predator risk using chemical cues and how prey responses to differing combinations of predator size and biomass propagate to lower trophic levels. The predation system consisted of adult and juvenile blue crabs, mud crabs, and their shared oyster prey. This intraguild (IG) crab predation system is ideal for examining size-based interactions because predation by crabs is crush-limited, predator and prey size determine predation interactions, and because size classes co-occur. The IG predators in this system, blue crabs (*Callinectes sapidus*), are important consumers and scavengers of estuarine environments (Micheli 1997) and prey on a variety of bivalve and crustacean species (Fitz and Weigert 1991; Micheli 1997). The IG prey, mud crabs (*Panopeus herbstii*), are small cryptic xanthid crab predators that occupy oyster beds at high densities (Lee and Kneib 1994; Hollebone and Hay 2007) and prey on a number of bivalve species (Seed 1980; Gibbons and Castagna 1985; Bisker and Castagna, 1987). Blue crabs and mud crabs both utilize shared

oyster prey (*Crassostrea virginica*) and therefore may contribute to top down control of oyster reefs that provide a wide variety of ecological services (Newell 2004). Blue crabs readily eat mud crabs in the lab (Hill and Weissburg,Ch. 1) and xanthid crabs and other non-portunid crabs make up approximately 43% of the diet of blue crab (Fitz and Weigert 1991). Predation risk from blue crabs is size based; large adult blue crabs (>100mm carapace width; CW) are voracious predators on mud crabs, whereas small juvenile blue crabs (40-60mm CW) rarely present a threat to mud crabs greater than 15mm CW (Hill and Weissburg, Ch. 1). This suggests mud crab prey may gain information on predation risk by assessing blue crab body size (or biomass), and that changes in mud crab foraging behavior in response to blue crabs may, in turn, affect their predation rate on oysters. Although previous studies have documented that toadfish predators cause NCEs on mud crabs and oyster prey (Grabowski 2004), it is unknown if blue crabs produce similar effects and whether (or how) predator size and biomass modulates the response.

In order to examine the connections between predator size and biomass in risk assessment and the expression of NCEs, I compared the predation rates of mud crabs on oyster prey in conditions where information on potential predation risk was transmitted via chemical cues. I employed predator treatments designed to differentiate the effects of size vs. biomass. These experiments were performed both in the lab and in the field where natural flow environments could affect the perception of chemical cues and thus the outcome of the NCE. The goals of my study were to: a) investigate whether blue crabs affect the foraging behavior of mud crabs on oyster prey through NCEs; b) to examine the effects of predator traits (size, biomass) on the expression of NCEs propagated by chemical cues; c) to document whether NCEs occur in the field in natural

water flows, and; d) to characterize the flow environment under which NCEs occur and examine potential variation in NCEs due to differences in temporal flow parameters.

Methods

Animal Collection and Maintenance

All experiments were performed at the Skidaway Institute of Oceanography (SkIO), Skidaway Island, Georgia, USA. Both blue crabs and mud crabs were collected from Wassaw Sound and associated tributaries. Hatchery-reared oysters (10-16mm in length) were obtained from Bay Shellfish (Tampa, FL). All animals were maintained in covered outdoor flow-through seawater tanks at SkIO for a minimum of 48 hours before experiments began. Blue crabs were maintained on a diet of shrimp and/or clams and were fed an *ad libitum* diet of shrimp and oysters once a day for 48 hours prior to experiments. Mud crabs were maintained on a clam diet and were starved 48 hours prior to experiments.

Laboratory Mesocosm Experiment

In order to examine how potential NCEs on mud crabs and their oyster prey are modulated by either blue crab biomass or size, I monitored mud crab foraging on oysters in response to differing treatments of caged blue crab predators in laboratory mesocosms supplied with flow-through seawater. Mesocosms (0.7m x 0.4m x0.3m) consisted of artificial oyster reefs constructed over approximately 2.5 cm of sand and shell hash substrate supplied with flow-through seawater. Artificial reefs were constructed by gluing 10 sun-bleached oyster shells (obtained from natural shell banks) to create similar small clusters (approximately 6 cm in diameter), and then bundling clusters with rubber bands to build a reef (21 clusters per tank). One juvenile oyster (10-15mm in length) was

secured on the face of each cluster using cyanoacrylate glue (21 oysters per tank). Artificial reef structure allowed us to control the placement of juvenile oysters within reefs; natural heterogeneous clusters prevented us from minimizing variation between tanks and also changed the ability of mud crabs to access oyster prey. One liter of shell hash was scattered around reef edges to mimic the natural structure of the habitat. Twenty mud crabs (similar to natural size class densities- twelve 15-20mm; four 20-25mm; three 25-30mm CW; Lee and Kneib 1994) were then added to tank. One of four caged predator treatments was submerged into the tank once mud crabs were added; one large blue crab (>100mm CW; approximately 130-180g), one small blue crab (40-60mm CW; 8-20g), multiple small blue crabs (40-60mm CW; totaling 130-180g), and a no-predator control. Predator cages consisted of plastic containers (0.34m x 0.20m x 0.12m) with multiple holes drilled through the side and a vexar mesh cover in order to allow diffusion of chemical cues into mesocosms, but prevented blue crabs from having any direct contact with mud crabs. Blue crabs were fed an *ad libitum* diet of shucked oysters every day. This diet was chosen because oysters are common prey for both adult and juvenile blue crabs (Eggleston 1990b). Mud crabs were allowed to forage on oysters in mesocosms for 2.5 days. Oyster survival in the tank was recorded every 24 hours. The total number of oysters eaten was recorded at the conclusion of the experiment, as well as the number of surviving mud crabs. Due to limited mesocosm space, 3 replicates of each treatment were run at one time in a randomized block design, with 6 runs in all, totaling 18 replicates for each treatment. The percent of oysters eaten was arcsine transformed to meet assumptions of normality and analyzed by a two factor ANOVA for the effects of

run and predator size (Zar 1999). The number of mud crabs surviving also was analyzed via a two-factor ANOVA with predator size blocked by run.

Field Experiment

In order to examine the size- or biomass-dependent effects of both blue crabs on mud crabs in the field, I caged mud crabs onto artificially constructed oyster reefs in the intertidal zone at Priest's Landing, Skidaway Island, GA. This site is located along the Wilmington River entrance to Wassaw Sound and is characterized by a combination of *Spartina* grasses, loose cluster and fixed oyster reefs, and slow to moderate water velocities (3-10cm/s; Smee et al. 2010). Blue crabs and mud crabs are common predators of oysters at this site.

Experiments were performed on mud substrates in the lower section of the intertidal, approximately one tidal foot below the level of local oyster reefs. The foraging success of mud crabs on juvenile oysters within experimental cages was monitored in response to predator biomass treatments from lab experiments; one large blue crab (>100mm CW; approximately 130-180g), one small blue crab (40-60mm CW; 8-20g), multiple small blue crabs (40-60mm CW; totaling 130-180g), and a no-predator control. Because cage recovery was limited to a short period at low tide, three replicates were run at a time in a randomized block design. Each treatment cage within a block was approximately 5m apart and each experimental block was 30m apart (i.e. block site). Three runs were completed, totaling 9 replicates.

Experimental enclosure cages (0.7m x 0.7m x 0.3m) were constructed of polyvinyl chloride (PVC) frames and covered with vexar mesh (mesh size: $1cm^2$). An oyster reef was constructed on one side of the caged area from a combination of both natural reef

and artificial oyster clusters (Fig. 3.1). The majority of the reef structure was comprised of four natural sun bleached oyster clusters (~0.20m in diameter), which maintained the structure of oyster reefs while removing live organisms that could serve as an alternate food source. Artificial oyster clusters were used to control for the placement oysters within the cage and each cluster had four oysters (10-16mm length) secured to their face with cyanoacrylate glue. Within each enclosure (Fig. 3.1), four artificial clusters each were placed inside the reef (within the refuge) and outside the reef (outside the refuge) for a total of 16 juvenile oysters both inside and outside of habitat refuges. I estimated the level of refuge use by mud crabs by measuring the number of oysters eaten both inside and outside the refuge. This information provided insight on the risk assessment behaviors of mud crabs; higher numbers of oysters eaten outside refuge would suggest that mud crabs perceived the environment as less risky. Fifteen mud crabs (eight 15-20mm CW; four 20-25mm CW; three 25-30mm CW-similar to natural population size structure; Lee and Kneib 1994) were placed within the oyster reef refuge. Each mud crab was marked with paint to differentiate it from potential mud crab immigrants into the cages. The influence of predator chemical cues was examined by placing caged predators on either side of the oyster reef refuge so that predator cues were reaching the refuge in either direction of the tide. One predator cage was placed inside the main enclosure, among the artificial clusters outside of the main reef refuge. The second predator cage was placed on the other side of the refuge outside of the enclosure walls (Fig. 3.1). I thought this design was a reasonable compromise between two potentially counter-acting influences of the enclosure cage mesh, which can restrict cue exchange across the mesh, but result in greater mixing of cues passing through the mesh. As described below, flow

measurements suggest the cages did not strongly alter the hydrodynamic environment inside the cage.

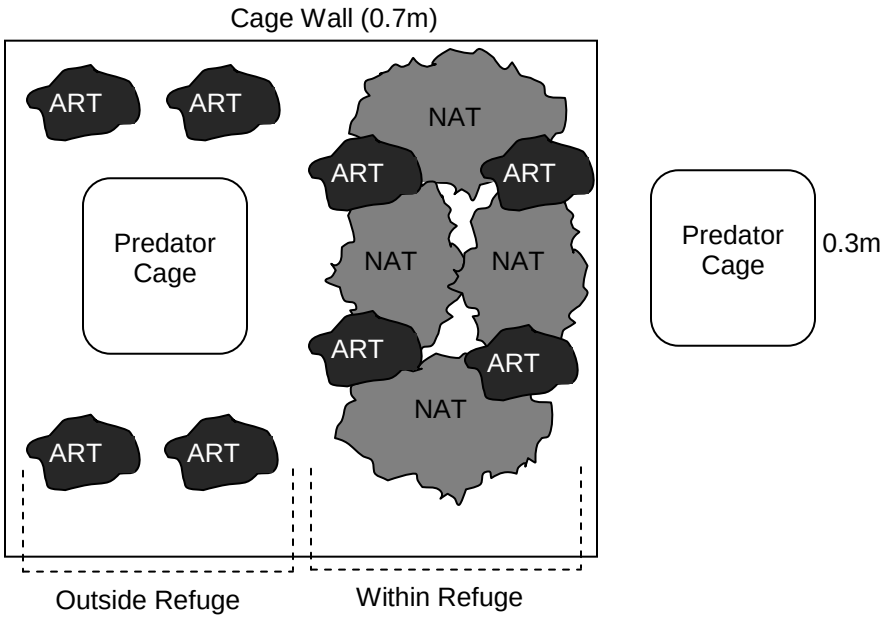

Figure 3.1. Diagram of caged reef design in field experiment. NAT= Natural dried oyster reef clusters; ART = Artificial oyster clusters with 4 attached live juvenile oysters. Oysters eaten on artificial reef among natural reef structure were designated as 'Within refuge predation'. Oysters consumed on artificial clusters separated from natural reef were designated `Outside refuge predation`.

Predator cages consisted of half-cylinder vexar cages (0.30m diameter x 0.30m long, mesh size: 1cm^2) placed on their side. Cage bottoms were covered with mesh screening to prevent blue crabs from accessing any mud crabs that sometimes sheltered under the cage as well as to prevent mud crabs from accessing predator food. All cages were secured to the substrate with rebar stakes. Predators were fed a diet of crushed oysters every day to ensure they were always producing cues. In addition, crab pots baited with three chicken necks were used to assess local abundance of blue crabs during each experimental run, as cues from mobile blue crabs could affect experimental outcomes.

The number of oysters remaining on each artificial cluster was counted every 24 hours for 48 hours. Any mud crabs found within cages and all oyster clusters were collected at the end of the 48 hour period, and were then transported back to the lab. Clusters were rinsed, and sorted for mud crabs to assure the counts of mud crabs were as accurate as possible. I determined the number of experimentally marked mud crabs as well as the number and carapace width of any immigrant mud crabs.

Preliminary analysis showed that the experimental run (i.e. time), but not block site had a significant effect on the percent of oysters eaten; thus, all data from sites were pooled and subsequent analysis was blocked by experimental run (time). The percent of oysters eaten after 48 hours was analyzed by a two factor ANOVA for the effects of run and predator size. The number of oysters eaten inside and outside the refuge was compared using a two-factor repeated measures (or split-plot) ANOVA with oysters eaten inside the refuge and outside the refuge treated as a within-block/repeated measures factor. The number of mud crabs (>15mm CW) found in the cage at the conclusion of the experiment (both experimental and immigrant crabs) was also analyzed by a two factor ANOVA. All mud crab immigrants above 15mm CW were included in this count as they also may have contributed to predation on oysters within cages (Hill, unpublished data).

Flow measurements in the field

Flow conditions vary over space and time in tidal-driven estuaries (Smee et al. 2010; Wilson et al. unpublished manuscript), and differing flow conditions could have significantly impacted the perception of chemical cues and thus the outcome of NCEs in each of the experimental runs. Consequently, I measured flow conditions over the course

of my experiments in an attempt to determine if any changes in NCEs observed throughout the field experiment could be attributed to changing flow characteristics. Flow conditions were monitored over the course of each experimental run using one acoustic Doppler velocimeter (ADV; Nortek). The flow was monitored in the same location and tidal height, over mud substrates and several meters away from experimental cages. The ADV was mounted so that flow measurements occurred at approximately 15cm over the substrate, and aligned so that the u-velocity component was in the primary tidal-flow direction. Flow velocities were measured at a frequency of 16Hz for 5 minutes every 15 minutes over the course of the experiment.

As my experiments were performed within cages, I performed additional measurements examining the effect of the cages on local flow parameters. Caging can often affect the magnitude of multiple flow parameters (Nowell and Jumars 1984; Miller and Gaylord 2007) and potentially inhibit (i.e increase turbulence) or facilitate (i.e. decrease velocity) the ability of animals to perceive cues. Flow measurements taken inside the cage were compared to those taken outside the cage at two locations for each experimental block position. One cage was modified by cutting a 10 cm swath from the top to allow for an ADV to be mounted in the center and measure flow velocities inside the cage. The cage was then secured with rebar stakes. Two other ADVs were mounted outside the cage; one 30cm directly upshore from the cage and the other 5m away at the same tidal height. These comparisons were repeated at the position of each block position of the predation trials in order to examine whether cage effects differ between blocks. All ADVs were mounted so that flow measurements occurred at 17cm above the substrate, slightly greater than mid height within the cage. Flow velocities were measured at a frequency of

16Hz for 5 minutes every 15 minutes for approximately 12 hours. From these velocity measurements both the total velocity and turbulent kinetic energy was calculated.

Total velocity (U) was calculated by the formula,

$U = \sqrt{u^2 + v^2 + w^2}$, where u, v, and w represent the velocity components x (along-stream), y (cross-stream), and z (vertical) directions. Turbulent kinetic energy (TKE) represents the magnitude of the velocity fluctuations (i.e. turbulence) that mix chemical cues and potentially decrease perception. TKE was calculated by the formula

$TKE = 1/2(\overline{u`^2} + \overline{v`^2} + \overline{w`^2})$, where u`, v`, and w' represents the magnitude of deviations of each velocity sample from the mean burst velocity in the x, y and z directions, averaged per burst. Data for total velocity and TKE over runs were unable to be transformed for normality. However as ANOVAs are typically robust for non-normal distributions (Zar 1999), I completed the analysis by a two factor ANOVA for run (1,2,3) and tide status (Incoming, Outgoing). I confirmed significance values for run and tide status using a one-way non-parametric Kruskal-Wallis (K-W). I also examined the effects of flow on the percent of oysters eaten using a two-way ANOVA for predator treatment and total velocity in which the average total velocity of each run was used as a measurement for each replicate site within that experimental run. To examine the effect of the cages on the flow environment, the total velocity and TKE were square root transformed to meet assumptions of normality and analyzed by a three-factor ANOVA for ADV position (inside the cage, outside the cage, 5m away from cage), Site (Block position 1,2,3), and Tide Status (Incoming, Outgoing).

Results

Lab Experiment

Chemical cues from blue crab predators had a significant biomass-dependent effect on the percentage of oysters eaten by mud crabs (Fig. 3.2; $F_{3,48}$=7.75, P<0.001). Cues from high biomass treatments (large and multiple small caged blue crabs) suppressed the percent of oysters eaten by mud crabs by approximately 20-25%. In contrast, single small blue crabs did not suppress mud crab foraging and were not significantly different from no predator controls (Fig. 2). Experimental run also affected the number of oysters eaten ($F_{5,48}$=5.41, P<0.002) but there was no run × predator treatment interaction ($F_{15,48}$=1.62, P > 0.l00). The number of mud crabs surviving the duration of the experiment varied significantly with run (N=18, $F_{5,48}$=3.02, P <0.02), but not by predator treatment ($F_{3,48}$=.73, P >0.50). I obtained the same results for ANOVA and post-hoc tests when the number of oysters eaten in each tank was corrected for mud crab survivorship by dividing predation by the number of surviving mud crabs (data not shown).

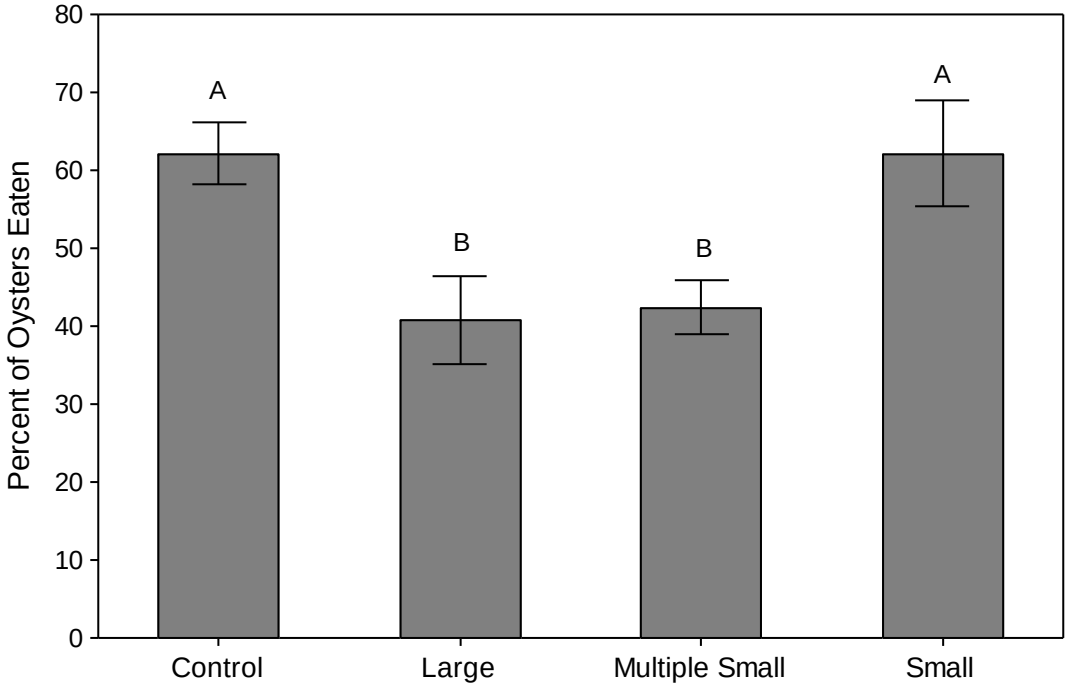

Figure 3.2. The percent of oysters eaten by mud crabs (mean ± SE) in response to predator biomass treatments in laboratory mesocosms. Different letters denote means that are significantly different based on Tukey post hoc tests (P<0.05).

Field Experiment

Cues from blue crab predator treatments significantly affected the percentage of oysters eaten by mud crabs (Fig. 3.3; N=9, $F_{3,23}$ =16.01, P <0.001), as did experimental run ($F_{2,23}$=5.06, P <0.015). Consistent with laboratory results, high biomass treatments (large and multiple small blue crabs) suppressed mud crab predation on oysters by approximately 50% relative to controls (Fig. 3.3). Oyster survival in response to cues from small blue crabs was not significantly distinguishable from the control. Analysis revealed no run × treatment interaction ($F_{6,23}$=0.75, P>0.600). The number of mud crabs recovered varied significantly with run ($F_{2,21}$=9.83, P <0.002) but not by treatment

($F_{3,21}$=0.97, P> 0.400). Approximately 65%, 50%, and 75% of mud crabs were recovered on Runs 1, 2, and 3 respectively. Despite differences in mud crab abundance, the ANOVA and post-hoc analysis were not different when correcting for the number of recovered mud crabs by dividing the oyster predation by average number of mud crabs recovered by run (data not shown). The number of blue crabs in the environment surrounding the cages was low, with less than 1 crab/trap/day caught on average. Thus, ambient crabs in the environment likely did not influence mud crabs within experimental cages.

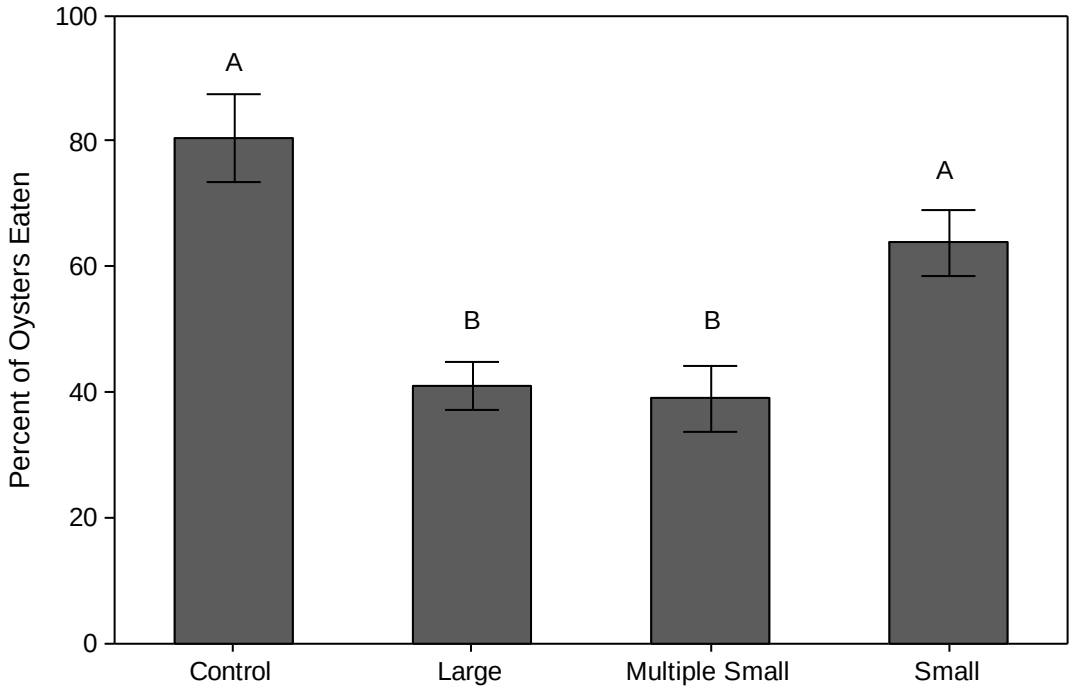

Figure 3.3. The percent of oysters eaten by mud crabs in field caging experiment (mean ± SE) in response to differing predator biomass. Different letters denote means that are significantly different based on Tukey post hoc tests (P<0.05).

As the amount of foraging outside the refuge may indicate the perception of risk by mud crabs (i.e. increased predation outside the refuge indicates a low risk environment), a repeated measures (or split-plot) ANOVA was used to analyze differences in the number of oysters eaten inside and outside the refuge (within-subject factor). Both blue crab predator treatment (Fig. 3.4; $F_{3,23}$=16.23, P <0.001) and run ($F_{2,23}$=4.93, P<0.02) significantly affected the number of oysters eaten inside and outside the refuge, but there was no predator treatment × run interaction ($F_{6,23}$=0.812, P > 0.50). Predation on oysters was significantly different as a function of position (inside or outside refuge; $F_{1,23}$=120.39, P <0.001) but the magnitude of this effect was dependent upon the predator treatment (Position×Treatment;$F_{3,23}$=8.24, P<0.002) and run (Position × Run; $F_{2,23}$=5.39, P<0.02). The greatest amount of predation in either position occurred in no predator controls and there was no apparent preference for oysters inside or outside of refuges (Fig. 3.3). Mud crabs showed a small preference for oysters within the refuge in response to single small blue crabs, but predation rates were high in both positions, averaging eight and twelve oysters eaten outside and inside the refuge (of 16 total in each position). Mud crabs strongly preferred to forage within the refuge in the presence of high biomass treatments (large and multiple small blue crabs); on average, only three oysters were consumed outside reef refuges whereas approximately 10 were consumed inside of the refuge (Fig. 3.4). The full interaction term was not significant (Position × Treatment × Run $F_{6,23}$=1.62, P > 0.10).

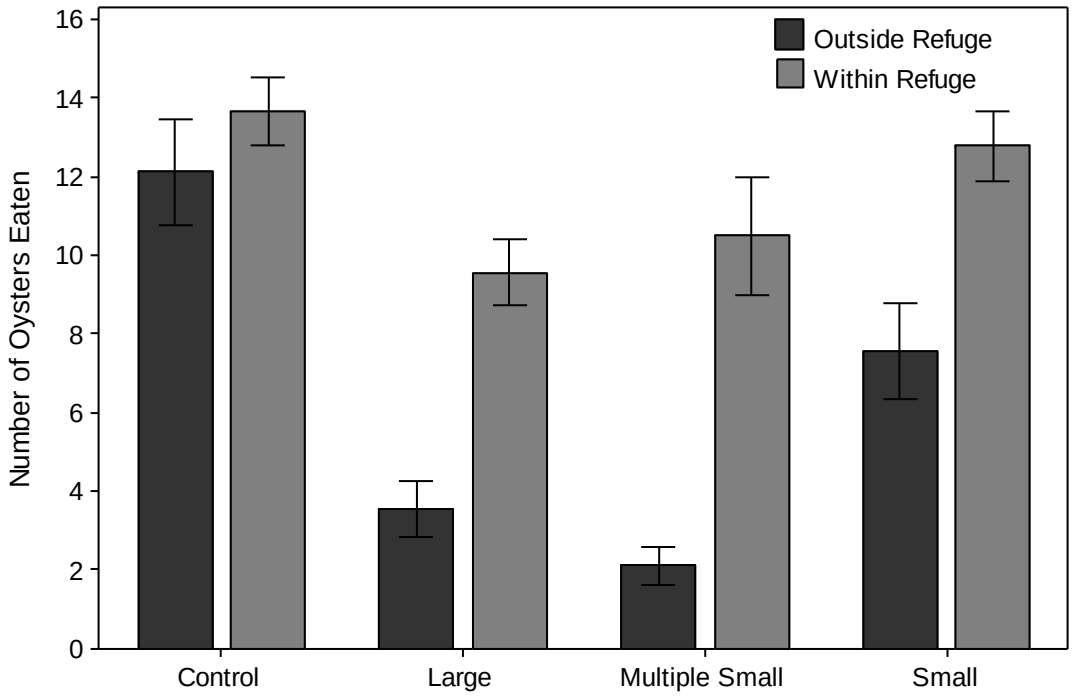

Figure 3.4. The number of oysters eaten outside (dark grey) and within (light grey) the refuge (mean ± SE). High numbers of oysters eaten outside the reef refuge indicate low risk environments where mud crab movement and foraging was not suppressed. The number of oysters eaten inside and outside the reef refuge (16 total in each area) are significantly different (Reef Position; $F_{1,23}$=120.39, P<0.001) and the difference between them is affected by predator treatment (Reef Position ×Predator Treatment; $F_{3,23}$=8.24,P<0.002).

Flow measurements

The ranges of both the velocity and TKE generally overlapped over the course of the experimental runs indicating that organisms experienced similar conditions (Fig. 3.5). Total velocity, but not TKE, was significantly different over the course of experimental runs (Fig. 3.5; ANOVA $F_{2,386}$=11.49, P<0.001, K-W P=0.005; ANOVA, $F_{2,386}$=2.96, P=0.053; K-W P=0.401 for velocity and TKE, respectively). Both total velocity ($F_{1,386}$=153.09, P<0.001; K-W P=0.005) and TKE($F_{1,386}$=20.73, P<0.001; K-W P<0.001) were significantly higher on outgoing tide vs. the incoming tide (Fig. 3.5). The

difference in total velocity was more pronounced in Run 3 where outgoing tide velocities were higher than the previous two runs (Fig. 3.5a; Run × Tide Status, $F_{2,386}$=6.66, P<0.002). As the significant changes in total velocity across the experimental runs could have contributed to differences in NCEs over time, I examined the effect of velocity and predator treatment in a two-way ANOVA. Although predator treatment was significant ($F_{2,23}$=12.13, P<0.001), neither the effect of total velocity ($F_{2,23}$=0.52, P >0.50) or the flow × treatment interaction were significant ($F_{6,23}$=0.66, P > 0.60). Consequently, velocity differences between runs did not alter overall magnitude of predation, nor did they differentially affect responses of mud crabs in blue crab predator treatments that delivered chemical cues.

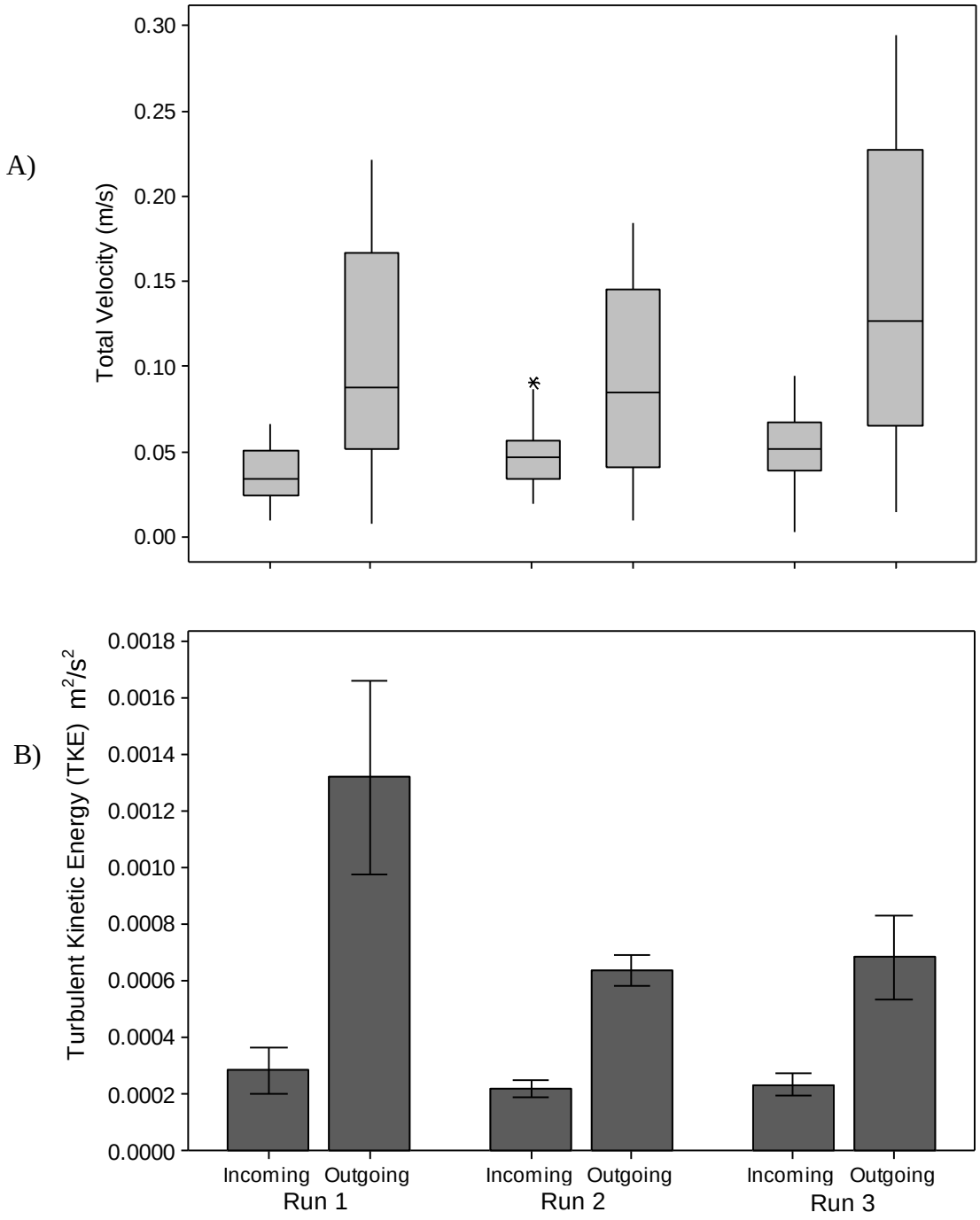

Figure 3.5. Environmental flow characteristics measured during experimental runs in the field. A) Box plot of total velocity. Each box represents the median, upper, and lower quartiles of data distribution. Whiskers represent 95% confidence intervals and * denotes outlier that was not excluded in analysis. B) Turbulent kinetic energy (TKE; mean ± SE).

ADVs placed in and around cages characterized the flow environment at the site of each experimental block. Unfortunately, beam correlation values were less than 60% (i.e. an object was blocking the sensor) for one of the ADVs at Site 1 so data from Site 1 was excluded. Flows were slightly, but significantly, modified by cage walls. Analysis of flow data by ANOVA revealed that cages significantly dampened the total velocity (Fig. 3.6a; $F_{2,189}$=11.32, P<0.001) but the magnitude of the dampening tended to be affected by flow velocities related to tide status (ADV Position × Tide Status, $F_{2,189}$=2.28, P=0.105). Flow velocity was generally greater by ~4-6 cm/s on the outgoing vs. incoming tide (Tide Status, $F_{1,189}$=88.90, P<0.001). During the higher velocities of outgoing tides, cage mesh dampened velocities to a greater extent than on the incoming tide. Velocities were dampened ~1cm/s on the incoming tide but ~3-4cm/s on the outgoing tide. Total velocities were also significantly impacted by block site ($F_{1,189}$=9.63, P<0.003). However, this difference reflects taking measurements on different days during which tide was transitioning from spring to normal tide levels. Despite these differences, the ranges of velocities in each ADV position overlap indicating that similar velocity fluctuations were occurring inside cages.

Similar to total velocity, TKE was higher on outgoing tide than incoming tide (Fig. 3.6b; Tide Status, $F_{1,189}$=10.15, P<0.003). Cages did modify turbulence levels ($F_{2,189}$= 5.73, P<0.005) but, like velocity, this effect was dependent on the tide status (Tide Status × ADV Position, $F_{2,189}$=6.39, P<0.003). Cage effects on TKE tended to act opposite to the effects of cages on velocity. Cages increased TKE a very small amount during incoming tides when turbulence levels were generally low. Cages did not affect turbulence levels

during outgoing tides when turbulence was generally higher. TKE was not significantly different based on site ($F_{1,189}$=0.62, P >.40).

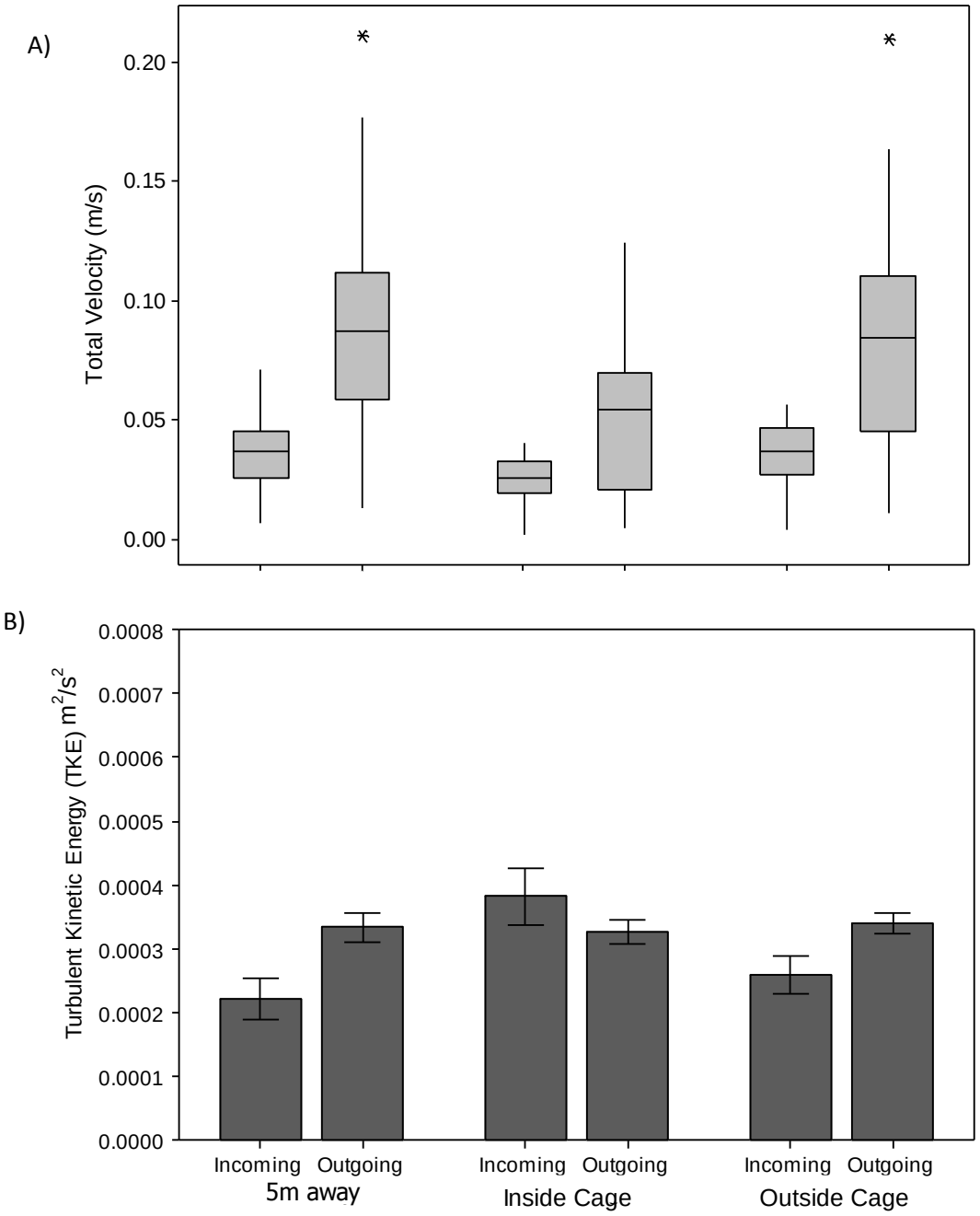

Figure 3.6. Flow characteristics measured by ADVs placed inside, directly outside, and 5m away from cages. A) Box plot of total velocity; each box represents the median, upper, and lower quartiles of data distribution. Whiskers represent 95% confidence intervals and * denotes outliers that were not excluded in analysis B) Turbulent Kinetic Energy (TKE; mean ± SE).

Discussion

Body size is increasingly recognized as an important factor in structuring ecological communities (Werner and Gilliam 1984; Rudolf 2006; Shackell et al. 2010; Rudolf and Lafferty 2011). My results extend the importance of this concept by indicating that predator body size and density can significantly impact the magnitude of NCEs through differential behavioral responses to predator biomass. These responses include changes in both the overall intensity of predation, as well as refuge use.

In my experiment, high biomass predator treatments composed of large and multiple small blue crabs resulted in increased oyster survivorship in both laboratory mesocosms and in the field (Fig. 3.2; Fig. 3.3). This increase in oyster survival is likely driven by mud crabs decreasing their movement, foraging activity, and patterns of distribution (Grabowski and Kimbro 2005; Hill and Weissburg unpublished data). This antipredator behavior in response to high biomass predator treatments caused mud crabs to alter patterns of habitat use and consume fewer oysters outside of reef refuges in comparisons with controls (Fig. 3.4). In contrast to the effects of high predator biomass, low predator biomass (i.e. a single small blue crab) did not significantly alter the number of oysters eaten relative to control cages in the lab or in the field (Fig. 3.2; Fig. 3.3). Patterns of predation outside of refuge habitats also indicate that compared to a high biomass treatments, perception of risk in response to low predator biomass was low; mud crabs were more likely to forage outside of reef refuges in the presence of a single small blue crab in comparison to high predator biomass treatments (Fig. 3.4).

The biomass specific behavioral response allows mud crabs to respond with risk appropriate behaviors to individuals of the two predator size classes. Large blue crabs are

high risk predators able to consume large numbers of mud crabs especially in low habitat complexity environments (Hill and Weissburg, Ch. 1). In contrast, small blue crabs were a low risk predator, rarely preying on mud crabs that were greater than 15mm CW. Mud crab activity was suppressed by caged single large, but not single small blue crabs. However, by increasing the predator biomass by raising the number of small blue crabs, mud crabs can be "tricked" into perceiving risk from these non-risky predators. This result suggests that the perception of risk is related to either chemical cue flux or concentration, as opposed to differences in chemical composition. Whether small blue crabs produce NCEs under natural conditions is dependent on their distribution, but I know of no data suggesting small blue crabs in nature would be aggregated as in my cages.

The results of my study establish that biomass-specific risk perception introduces another important type of context-sensitivity in NCEs where body size, density, and aggregation will affect the expression of NCEs at least when risk perception is based on chemical cues. There are two important consequences. First, since concentration or flux of chemical cues is dependent on both the size of the individual predator and predator density, we may be misestimating both the magnitude and importance of NCEs in natural systems by assuming that all predators in a species have an equal effect, and by ignoring the degree of aggregation. For instance, highly aggregated small predators may have as great an effect as a single larger consumer, and be more likely to induce risk responses than small predators at lower densities. Unfortunately, many studies of chemically-mediated NCEs do not scale biomass of predator treatments to field conditions, much less evaluate the influence of different predator size distributions.

Second, chemical detection of size-based risk appears to operate differently when compared to other modalities, and suggests that the role of biomass in the expression of size-based NCEs may be modality specific. Previous studies have demonstrated that individual predator body size plays a role in risk perception in visually-oriented vertebrate predators (Chivers et al. 2001; Stankowich and Blumstein 2005, Rudolf 2006). For instance, larval spring salamanders (*Eurycea cirrigera*) decrease their activity levels in response to cohorts of large salamander predators (*Gyrinophilus porphyritcus*), but not to small individuals who are less likely to prey upon them (Rudolf 2006). The response to small predators does not differ if the predator density is increased, although it is unclear if the increase in predator density was enough to produce the cues of a large predator. Consequently, the sensory modality by which risk is determined may have large impacts on how predator size vs. biomass affects the expression of NCEs in natural communities.

My study also shows how NCEs are expressed in relatively unaltered field conditions, as opposed to more static field mesocosms or simplified laboratory settings where cues can saturate the water and are persistent. Although such studies are essential to document that NCEs can occur, these designs are often not representative of natural environments where water flow mixes and disperses odor cues (Weissburg, 2000; Webster and Weissburg 2001) and diminishes the potential for NCEs to be expressed. A limited number of prior studies in natural conditions (Powers and Kittinger 2002, Smee et al. 2008) indicate the fluid environment can change the scale and likelihood of prey perception of predator cues, and thus, the potential for NCEs to be expressed. In my study, I found that the field environment did not impede the NCEs between blue crabs and mud crabs as results were similar in both the lab and the field.

I characterized the flow environment to provide information on environmental conditions relative to the expression of NCEs as well as to determine whether differences in NCEs throughout the experimental runs could be attributable to changing flow regimes. Estuaries display considerable variation in hydrodynamic properties that change over both time and space (e.g. Smee et al. 2010, Wilson et al. unpublished manuscript). Not all conditions are permissive of prey perception (Smee et al. 2010), making it important to establish those situations in which NCEs do occur. I found that flow ranges were similar over the course of experimental runs, but the overall magnitude of total velocity was significantly different (Fig. 3.5). This is not unexpected as tidal flow is generally extremely variable over time due to differences in waves, tides, and wind. Despite these differences in flow characteristics, NCEs are still expressed over the course of the experiment and overall patterns of predation in response to blue crabs over the course of the experiment remained unchanged (data not shown). The magnitude of the NCE varies over the course of the runs, but is not associated with either TKE or total velocity suggesting that the difference in flow regimes over time did not produce variation in NCEs in this system. However in order to fully examine the interactions of flow and NCE, it may be more appropriate to sample NCEs on smaller time scales such as incoming vs. outgoing tide, where flow characteristics are significantly different (Fig. 3.5). In my experiment, it is possible that any effects of water flow on NCEs average out over several days. Differing sites may also help to further differentiate the effects of flow on NCEs. For instance, Smee et al. (2010) demonstrates differences in clam survival over multiple sites with differing flow characteristic suggesting that perceptive abilities can change across sites with different velocity and mixing levels. The faster sites examined

by Smee et al. (2010) had greater average velocities (>15 cm s-1) and turbulence level than seen in the present study, and differences across the study sites exceeded the greatest differences I see in my measurements. Further, flow measurements within and between sites in Wassaw Sound suggest that the flow parameters within sites separated by <10 m are highly correlated (Wilson et al., unpublished manuscript) suggesting that perceptive abilities of organisms may be more likely to vary between widely separated areas and not within sites.

Performing field experiments on NCEs requires cages that may alter the flow environment, but my results suggest we can perform the required manipulations in the field with only small effects. Cages increased TKE by a small, but significant magnitude during incoming tides when turbulence levels were the lowest (Fig. 3.6b). This increased turbulence within cages would disperse chemical cues inhibiting perception of predators and NCEs (Smee et al. 2008). In contrast, the impact of cages on water velocities may have increased perception of predators as cages generally dampened the total water velocity, possibly allowing cues to remain in cages for longer periods (Fig. 3.6a). This result is not unexpected as cages often impede water movement especially with smaller mesh sizes (Nowell and Jumars 1984; Miller and Gaylord 2007). However, it is important to note that although cages did affect water flow characteristics, both velocity and TKE still fell within ranges of those seen outside the cage. Thus, while I cannot exclude the possibility that the decreases in water velocity created by the cages may have biased my results toward a higher magnitude NCE, I am confident that NCEs would still be observed in this system under natural conditions.

In conclusion, these results demonstrate cues from blue crabs can suppress mud crab foraging on oyster prey, and affect the degree of refuge use. The magnitude of this NCE is significantly modulated by predator biomass where large risky predators and multiple small predators cause large suppressions of mud crab foraging and increased refuge use in contrast to effects seen in the presence of individual small non-risky blue crabs. Furthermore, these interactions occur in the field under natural flow characteristics suggesting NCEs in this system are important in the natural environment. This also demonstrates that fisheries managers may need to preserve body size distributions of fished species in order to maintain cascading interactions (Benoit and Swain 2008; Darimont et al. 2009; Shackell et al. 2009). Blue crabs are among many species currently experiencing shifts to smaller size distributions as a result of over exploitation (Lipcius and Stockhausen 2002). This alteration of size structure and its resulting impacts to NCEs may have significant negative consequences for oyster reefs that may be dependent upon blue crabs for top-down control of mesopredators, such as mud crabs. Future efforts should further examine the role of consumers and their body size on NCEs in order to determine the full impact to fished species and estuarine communities. In the future, it may be necessary to protect species from overfishing to preserve the stability of food webs, but also to preserve their population size structure to maintain predator traits and cascading NCEs (Shackell et al. 2009).

CHAPTER FOUR

The effects of mud crab predation and long term non-consumptive effects (NCEs) on oyster recruitment and growth

Abstract

Extensive research has identified numerous abiotic processes that limit recruitment and survival of oysters (*Crassostrea* sp.). In contrast, the role of predation in limiting oyster recruitment is relatively understudied, despite the abundance of predator species which prey upon juvenile oysters. Furthermore, a number of recent studies suggest that non-consumptive effects (NCEs), often mediated by predator chemical cues, are important interactions in structurally complex habitats such as oyster reefs. Yet, to my knowledge no study has investigated whether NCEs can significantly increase oyster survival over long term natural recruitment cycles. My previous studies have demonstrated that chemical cues from blue crabs (*Callinectes sapidus*) decrease the foraging activity of mud crabs (*Panopeus herbstii*) resulting increased oyster survival. This suggests that trophic cascades between these species may be beneficial to oyster reefs. Here, I investigated the role of mud crabs and the chemical cues of blue crabs on the recruitment and survival of oysters in the field. Recruitment of oysters was extremely high in experiments and any evidence of predation or NCEs was effectively swamped by high oyster densities; although, this may have been a consequence of experimental design. In contrast, foraging mud crabs suppressed oyster growth which resulted in significant differences to oyster size class distributions. Consequently, mud crabs may increase the time period in which oysters are vulnerable to predation from a suite of predators. These

results indicate that the impacts of predation are likely highly dependent upon the density of oyster recruits as well as the time period under which recruitment is considered.

Introduction

Post-settlement mortality driven by both biotic and abiotic processes can significantly affect invertebrate recruitment and the structure of benthic communities (as reviewed by Hunt and Scheibling 1997). Although many studies have recognized the abiotic mechanisms which limit recruit survival, such as thermal stress and disturbance, the conclusions on the role of biotic influences, such as predation, on recruitment success are often inconsistent and system dependent. Further, many of these studies only examine direct consumptive interactions. For instance, several studies by Osman and Whitlach (1995, 1998, 2004) demonstrated predation by mobile fishes and invertebrates controls whether communities are dominated by ascidians or bryozoans. In contrast, Sams and Keough (2007) determined that predators had relatively little effects on most sessile invertebrate species. Carrol (1996) determined that predators could decrease sessile invertebrate recruitment at low recruit densities, but that predators were ineffective at high recruit densities which effectively swamped predators. Thus, the effects of predation on recruitment survival and community structure may be context dependent on the system or recruit and predator density.

Oysters (*Crassostrea sp.)* have been extensively studied in terms of abiotic processes that predict larval settlement and also limit recruit survival such as hydrodynamics (Bushek 1988), thermal stress (Lutz et al. 1970), and tidal height (O'Beirn et al. 1995, Bartol et al. 1999). However, even though oyster reefs provide habitat to numerous bivalve predators, the role of predation in limiting recruiting oyster populations has been highly under-

investigated (but see Anderson and Connell 1999, Newell et al. 2000, O'Connor et al. 2008).This paucity is especially significant as increased oyster recruitment helps to renew and build oyster reefs that are currently declining in abundance in many parts of the U.S. (Beck et al. 2011). Mud crabs (*Panopeus herbstii*), blue crabs (*Callinectes sapidus*), stone crabs (*Menippe mercenaria*), toadfish (*Opsanus tau*), and black drum (*Pogonias cromis*) have all been implicated as predators on juvenile oysters (McDermott 1964, Bisker and Castagna 1987, Eggleston 1990, Abbe and Breitburg 1992, Brown and Haight 1992, George et al. 2008). Further, oyster settlement is increased when settlement surfaces are enclosed by mesh suggesting that protection from predators can enhance oyster recruitment (O'Beirn et al. 1996). However, few studies have addressed the role of predators in mortality of oyster recruits. Exclusion of toadfish predators resulted in increased recruitment and survival of oyster populations in Australia (Anderson and Connell 1999). O'Connor (2008) found that various combinations of crab predators (stone crabs, mud crabs, and blue crabs) reduced the recruitment of oysters; however the mechanism for the differential recruitment (i.e. decreased settlement or predation) was unknown. Consequently the role of predation in post-settlement oyster mortality is still largely undetermined.

Additionally, several studies in oyster reef communities have been highly influential in determining the role of non-consumptive effects (NCEs) in shaping community structure (Grabowski 2004, Grabowski and Kimbro 2005, Ferner et al. 2009, Hill and Weissburg Ch. 3). Non-consumptive effects are defined as changes in prey behavior or refuge use in response to predators which result in a variety of direct and indirect effects (Werner and Peacor 2003). For instance, Grabowski (2004) demonstrated that although toadfish

predators cannot access mud crabs in complex reef structure, toadfish suppress mud crab foraging on oysters occur even within oyster habitat refuges. Chemical cues from these predators are a likely mechanism, and other experiments between blue crabs and mud crabs have documented similar interactions. For instance, chemical cues from blue crab predators suppress mud crab foraging and reduce movement of mud crab between reef patches (Hill and Weissburg Ch 2, Hill and Weissburg Ch. 3) As a result, non-consumptive effects are believed to influence prey survival more than consumptive predatory interactions in complex oyster reef habitats (Grabowski 2004, Grabowski and Kimbro 2005). Although such studies establish the potential importance of NCEs in oyster reef communities, they occur over very short time scales at limited prey densities and thus fall short of demonstrating whether NCEs are important in predicting long term oyster survival. Longer term experiments are needed in order to demonstrate that short term responses to predator pulses are representative of longer term predator-prey dynamics when prey are constrained by other aspects of their life history (Werner and Peacor 2003, Bolker et al. 2003).

I conducted a field experiment to address whether predator-driven consumptive effects and NCEs impact the survival, growth, and long term recruitment success of oysters in natural habitats. In order to examine these interactions, I investigated the role of mud crab predation (*Panopeus herbstii*) and the long term NCEs from the blue crab (*Callinectes sapidus*) on the recruitment of oysters (*Crassostrea virginica*). Mud crabs occupy oyster beds at high densities (Lee and Kneib 1994, Hollebone and Hay 2007) and can prey on numerous bivalve species at high rates, including oysters (Seed 1980, Bisker and Castagna 1987, Hill and Weissburg Ch. 1). Mud crab predators recently have been

implicated in a variety of trophic cascades in estuarine environments and are thus expected to highly impact oyster reef community structure (Grabowski and Kimbro 2005, Grabowski et al. 2008, Hill and Weissburg Ch. 1). Blue crabs are mobile portunid crabs that significantly affect the survival of multiple estuarine species such as bivalves and various crustaceans including mud crabs (Eggleston 1990a, Eggleston 1990b, Micheli, 1997; Fitz and Weigert 1991). Furthermore, short term experiments have documented a NCE between mud crabs and blue crabs which increases oyster survival (Hill and Weissburg, Ch. 2, Ch. 3). Thus, although blue crabs are oyster predators, they also can have positive indirect effects on oyster survival by controlling mud crab populations through consumptive and NCEs. The objectives of my study were to a) determine if predation by mud crabs affects the recruitment of oysters through differential survival and growth and b) to examine whether NCEs caused by blue crab chemical cues successfully impact mud crab predation and increase oyster recruit survival over long time scales.

Methods

Study Site

This experiment was performed next to oyster reefs in the Wilmington River at Priest's Landing, Skidaway Island, Georgia, USA. This site is directly adjacent to Wassaw Sound and is composed of both *Spartina* grasses and intertidal oyster reefs. Oysters commonly settle in this area in moderate to high densities (O'Beirn et al. 1995, Hill, *personal observations*) and both blue crabs and mud crabs are common predators at this site. This experiment ran from early June to early August 2009 which encompasses

periods of high oyster recruitment and growth (Michener and Kenny 1991, Knights and Walters 2010)

Experimental Design and Field Methods

In order to examine the effects of mud crabs and NCEs on oyster recruitment success, I placed baskets of oyster shell next to oyster reefs and measured the recruitment, survival, and growth of oysters in response to differing predator manipulations. This method was chosen as bagged oyster shell is commonly used to restore oyster reefs (O'Beirn et al. 1996). Oyster baskets consisted of plastic storage baskets (0.35m x 0.25m x 0.12m) with vexar mesh lids (mesh size 1cm^2) containing approximately 20-23 oyster shells. The number of oyster shells constituted a total surface area (SA) of 800cm^2 (±5cm^2) as determined by using the length (L) and width (W) of shells to approximate ellipse surface area (SA) by the formula $SA = \pi \frac{1}{2}(LW)$. Cylindrical predator cages (0.30m diameter x 0.30m long; mesh size 1cm^2) were then secured to either side of all baskets using zip ties. Each basket was then assigned to one of four predator treatments: 1) Foraging mud crabs 2) Blue crab predator cues 3) Foraging mud crabs + blue crab predator cues 4) No predator control. Each treatment had a total of ten replicates. Treatments of foraging mud crabs consisted of four mud crabs (20-25mm carapace width; CW) which were placed within oyster baskets where mud crabs could access and prey upon settling oysters. Blue crab chemical cues were created by placing one blue crab (>100mm CW) in each predator cage attached to oyster baskets. This arrangement ensured that chemical cues would enter the basket in either direction of the tide. Finally, all baskets and cages with predators received small sponges to prevent desiccation at low tide.

Baskets of oysters and predators were assembled and transferred to the field where they were placed in a randomized block design within naturally occurring oyster reef. This placement facilitated natural oyster recruitment as oysters exhibit gregarious settlement in response to conspecific cues (Turner et al. 1994). Five replicates were placed on either side of Wilmington River. Each treatment basket was separated by approximately 3-5m and each block was separated by 5-10m depending on the distance to the adjacent oyster bed. In order to protect oysters from high sedimentation rates that can lower oyster survival, baskets and attached predator cages were suspended above the substrate by approximately 20cm using polyvinyl chloride (PVC) pipe that was hammered into the substrate. Sediment also was periodically rinsed from oyster baskets. Blue crabs were fed a diet of clams (*Mercenaria mercenaria*) every few days in order to ensure that they were producing cues. Mud crabs also were fed a small amount of chopped clams a few times a week in order to prevent starvation, especially in the early weeks of the experiment when no oysters were present. Any dead blue crabs in predator cages were replaced during these times. The number of mud crabs within baskets was checked every two weeks and missing mud crabs were replaced and immigrant mud crabs were removed. Immigrant mud crabs were easily distinguished from experimental crabs due to their smaller body size (8-15mm CW).

All baskets were removed from the field after approximately two months, at which point there was a high density of juvenile oysters within baskets, as well as evidence of predation on oysters (i.e. oyster scars). I recorded the number of immigrant mud crabs in baskets and oyster shells were bagged and frozen for later analysis.

Oyster Recruitment Measurements and Analysis

Oysters are particularly unique and ideal to study post-settlement mortality because when they die or are consumed by predators the lower valve of the oyster shell remains on the settlement surface creating a 'scar' that indicates the successful settlement of an individual (Anderson and Connell 1999). This aspect allows us to differentiate the number of oysters that recruit (all individuals-both scars and oysters) from the amount that survive as there were no scars on oyster shell prior to field deployment. As such, I assessed the total amount of recruitment, which was composed of both the total number of surviving oysters as well as the number of oyster scars. Since mud crabs (20-25mm CW) may prefer oysters between 4-8mm (Bisker and Castagna 1987), I also tallied the number of oysters that were greater than 5mm in length as the abundance of larger oysters may be more indicative of mud crab predation patterns. Following this same logic, I also recorded the number of scars that were greater than 5mm in length in an attempt to separate background mortality from predation. The length and width of all oysters over 5mm was also measured in order to determine whether oyster growth was affected by predator treatment.

A total of 8 replicates were measured for the number of oysters and scars and a total of 7 replicates had all oysters greater than 5mm measured. The remaining replicates were not measured as power analysis on data variance revealed that additional replicates would not yield enough statistical power to differentiate treatment groups. The total amount of recruitment (i.e. no. scars + no. oysters) and number of oysters, scars, and scars greater than 5mm were analyzed by two-way ANOVA for the effects of experimental block and treatment. The number of oysters greater than 5, 10, and 15mm were log transformed to

meet assumptions of normality and analyzed by two-way ANOVA for the effects of block and predator treatment. If blocking factors were not significant, data was pooled and analyzed by a one-way ANOVA for predator treatment.

Results

On average, 1,500-2,000 oysters recruited to oyster shells within field baskets (Fig. 4.1). The total amount of recruitment was significantly different in each experimental block ($F_{7,21}$=3.73, P=0.009) but was not significantly affected by predator treatment ($F_{3,21}$=0.93, P=0.442). Generally, between 800 and 1200 of these oysters survived (Fig. 4.2). The lowest amount of oysters surviving in baskets was generally found in mud crab+blue crab cue treatments (Fig. 4.2). However, the survival of oysters was not significantly different based on predator treatment ($F_{3,28}$=0.79, P=0.509).

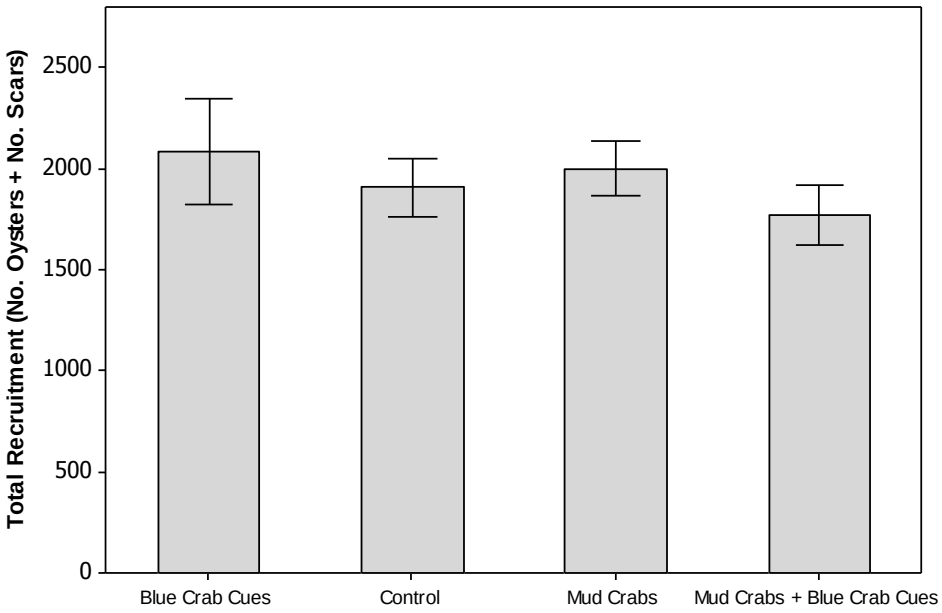

Figure 4.1. The number of oysters recruiting to oyster shells per basket (mean ± SE). The total recruitment is equal to the total number of oysters and scars observed on oyster shell and is not significantly affected by predator treatment ($F_{3,21}$=0.93, P=0.442).

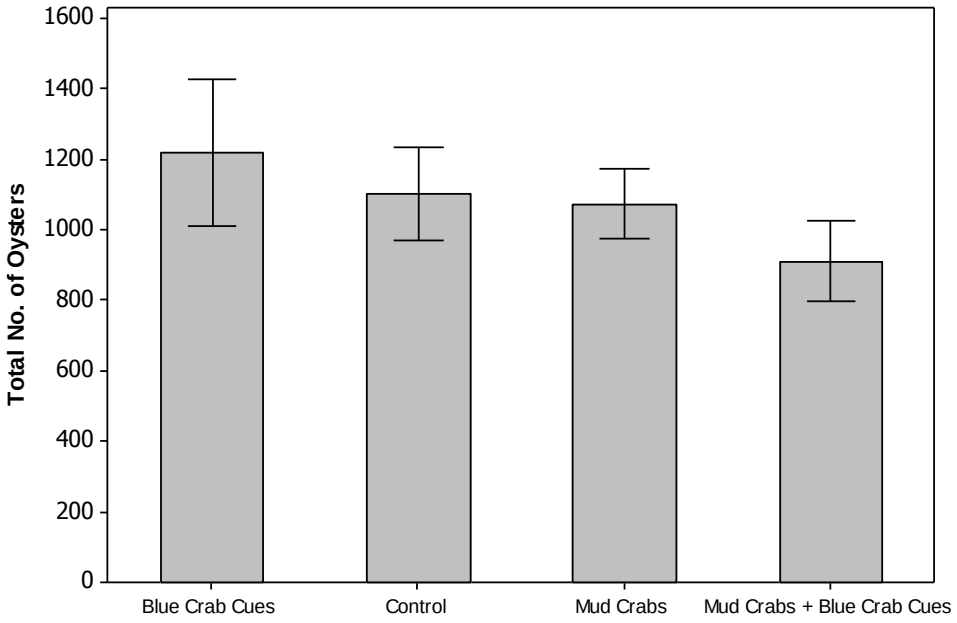

Figure 4.2. The number of surviving oysters per basket (mean ± SE). The number of surviving oysters is not significantly different in predator treatments ($F_{3,28}$=0.79, P=0.509) suggesting that predation is not influencing oyster recruitment.

Additionally, oyster death as indicated by scars was not significantly different by predator treatment ($F_{3,28}$=0.37, P=0.773). On average between 750 and 900 scars, which are indicators of both predation and background mortality, were observed on oyster shells (Fig. 4.3). However, there was a trend for mud crab treatments to have slightly higher mortality (~900 scars) than other predator treatments. In attempt to isolate predation events from background mortality, I examined the number of scars >5mm. Although foraging mud crab treatments again generally had a greater number of scars (Fig. 4.4), approximately 50 scars more on average than other treatments, the number of scars was not significantly different based on predator treatment ($F_{3,28}$=1.48, P=0.241). The largest amount of scars >5mm were found in mud crab treatments, followed by mud crab+blue crab cues, and then control and blue crab cues. Power analysis indicates that based on

current variation a total of 25 replicates would be needed in order to have enough power (β=0.8) to avoid type II error.

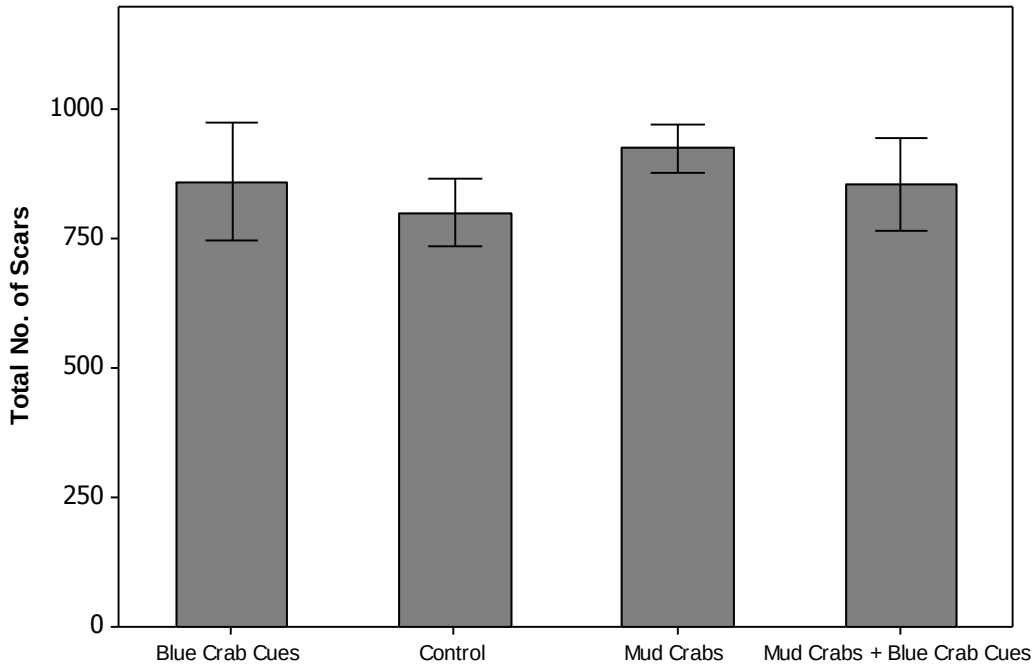

Figure 4.3. The total number of oyster scars on oyster shells per basket (mean ± SE). The number of scars is not significantly affected by predator treatment ($F_{3,28}$=0.37, P=0.773) suggesting that predation was not distinguishable from background mortality.

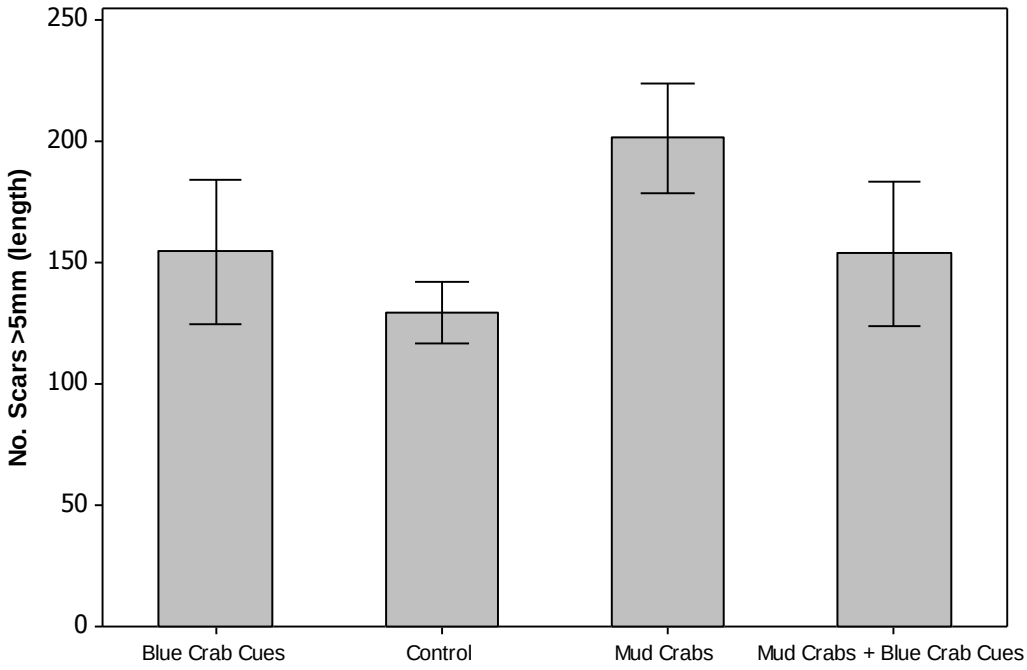

Figure 4.4. The total number of scars that were greater than 5mm in length per basket (mean ± SE). Scars greater than 5mm in length were more likely to indicate predation events. However, the treatment groups are not significantly different ($F_{3,28}$=1.48, P=0.241).

In order to examine whether predator treatments affected oyster body size distributions, either through differential survival or differences in oyster growth, I recorded and measured all oysters greater than 5mm in length. The number of oysters >5mm in length was not significantly different based on predator treatment (Fig. 4.5; $F_{3,23}$=1.09, P=0.374). However, oyster size class size distributions were significantly affected by some treatments when I excluded oysters <10mm in length (Fig. 4.5). The number of oysters >10mm in length was significantly different in differing predator treatments ($F_{3,23}$=4.2, P=0.017). There were less than 100 oysters >10mm in both treatments containing mud crabs, but approximately 1.5-2× as many oysters in control and blue crab cue treatments, respectively (Fig. 4.5). The number of oysters >15mm also was significantly different based on predator treatment ($F_{3,23}$=7.28, P=0.001). Treatments of

blue crab cues had the highest abundances of >15mm oysters (~30 oysters), followed by controls (~20 oysters), mud crabs and mud crabs+blue crab cues (~5 oysters). These differences in oyster size class distributions could be explained by either a) differences in growth or b) preferential mud crab predation on larger size classes. In attempt to account for mud crab predation, I partitioned the total number of oyster recruits >5mm in length (i.e. the total number of oysters and scars >5mm) by predation (scars) and survivors (oysters; Fig. 4.6). If mud crab predation caused lower abundances of large oysters, the total abundance of oysters (i.e. oysters and scars) across all treatments would be similar; although scars would make up a greater proportion of recruitment in mud crab treatments. However, this pattern is not supported by the data. In contrast, the total number of recruits >5mm in both control and blue crab cue treatments was still substantially higher (~1000 oysters) than mud crab treatments and scars did not make up a greater proportion of recruitment in treatments with foraging mud crabs (Fig. 4.6). This alternate pattern suggests that growth, and not differential predation, results in differing oyster size class distributions.

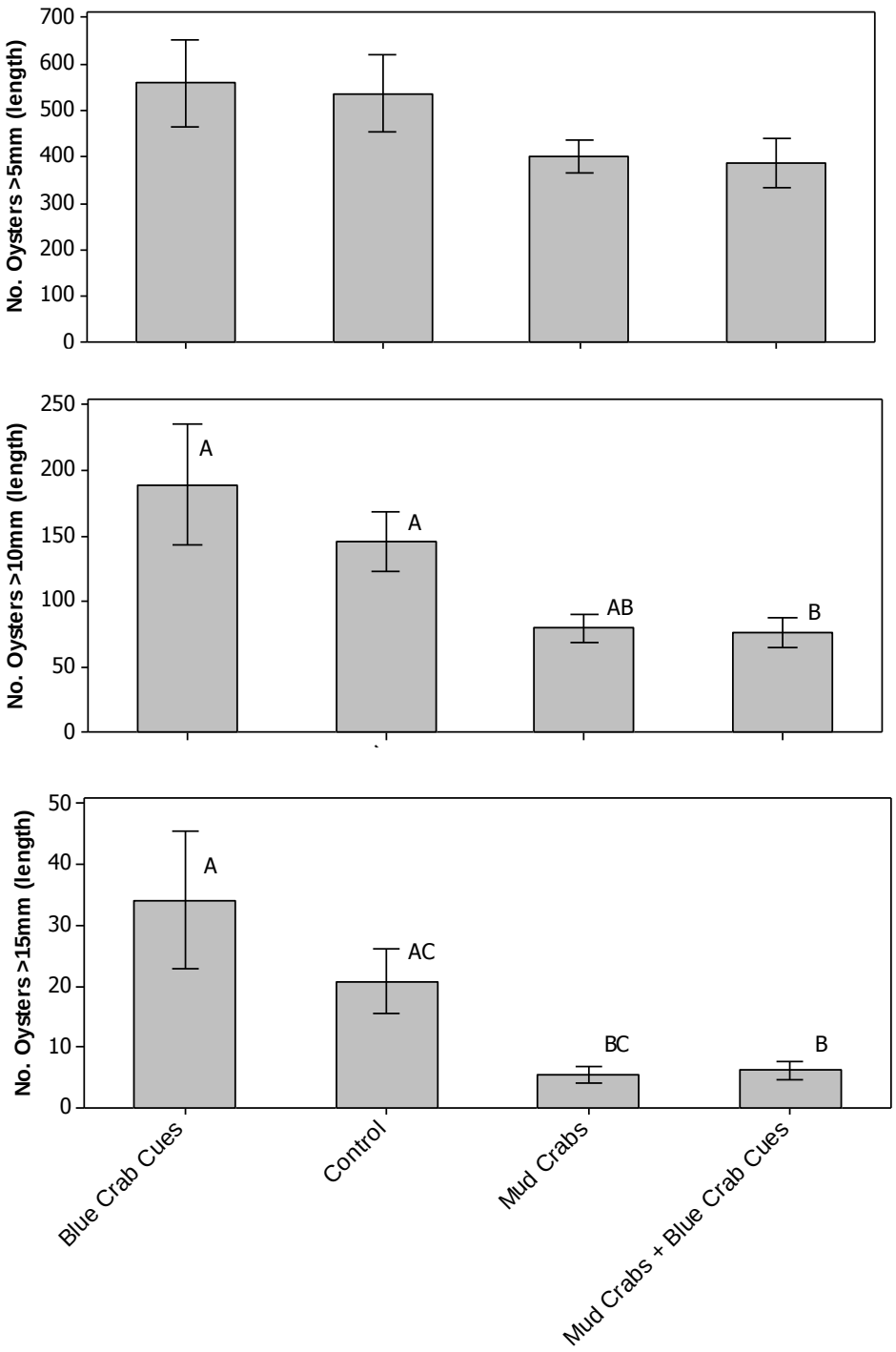

Figure 4.5. The number of surviving oysters differentiated by size class (>5,>10, >15mm; mean ± SE). These size classes reflect differences in oyster growth or survival. The number of oysters >5mm in each predator treatment was not significantly different ($F_{3,23}$=1.09, P=0.374). However, the number of oysters >10mm and >15mm were both significantly different based on predator treatment ($F_{3,23}$=4.2, P=0.017; $F_{3,23}$=7.28, P=0.001). Different letters denote means that are significantly different based on Tukey post hoc tests (P<0.05).

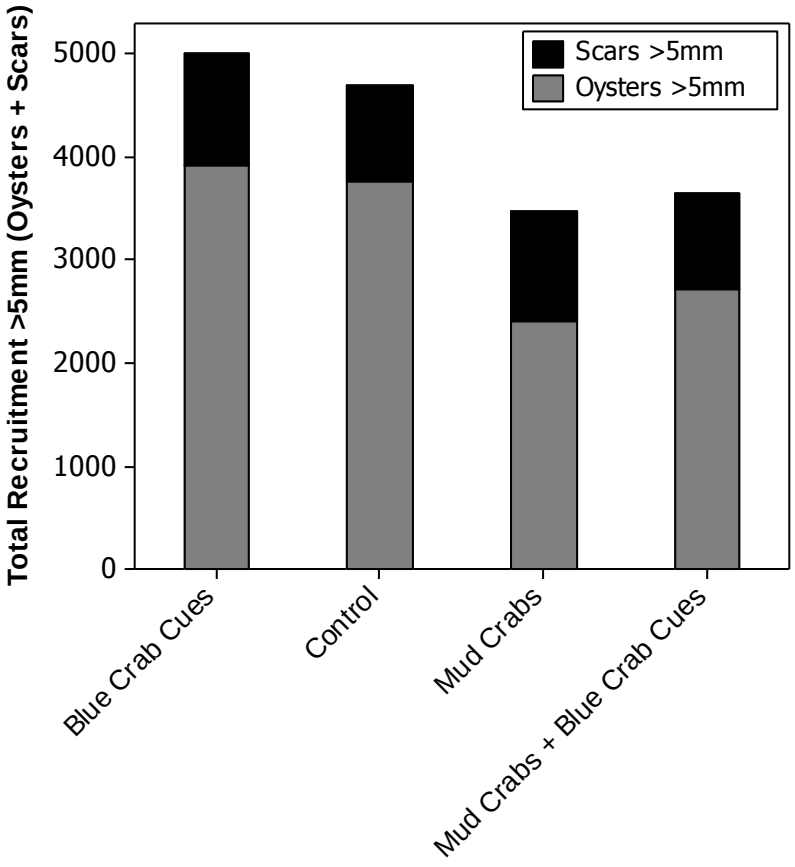

Figure 4.6. Proportion of total (i.e. combined replicates) oyster recruitment >5mm attributable to predation (i.e. scars). Differential abundance of oyster size classes is not explained by patterns of predation in predator treatments.

Discussion

Although many recent studies indicate that mud crabs significantly reduce the survivorship of juvenile oysters (Grabowski 2004, Grabowski 2008, Hill and Weissburg Ch. 1, Hill and Weissburg Ch. 3), my results indicate that this predation is not intense enough to significantly affect the recruitment of oysters onto reefs. Oyster recruitment was extremely high during the experiment where thousands of oyster spat recruited onto shell within oyster baskets (Fig. 4.1). Although oyster prey was plentiful, the presence of mud crabs did not significantly alter the number of oysters surviving suggesting that mud

crab predation on oysters was low (Fig.4.2). In addition, the number of scars indicating oyster death was not significantly different in treatments with and without foraging mud crabs supporting the evidence that mud crab predation was not distinguishable from background mortality (Fig. 4.3). Both the high numbers of oyster recruits and the lack of measurable mud crab predation suggest that oysters at this location effectively swamp mud crab predators (Underwood and Keough 2001) and mud crabs are not effective at limiting high densities of recruiting oyster populations.

As mud crab predation rates were low in general, the NCEs of blue crab cues on mud crabs did not appear to play a significant role in this experiment. In previous experiments, the application of blue crab chemical cues increased oyster survival by lowering mud crab foraging rates (Hill and Weissburg Ch. 2, Hill and Wiessburg Ch. 3). There is some evidence that blue crab cues lowered mud crab predation rates. Both the total number of scars (Fig.4.3) and the number of scars >5mm (Fig. 4.4) are greater in mud crab treatments than in mud crabs foraging in the presence of blue crab cues suggesting mud crabs reduced their foraging in response to blue crabs Although this trend is not significant, it does give some indication that blue crab chemical cues may have affected mud crab predation. However, the differences in predation were not significant enough to cause increased oyster survival in mud crab+blue crab cue treatments (Fig. 4.2). If this experiment had run for a longer time period allowing for increased predation by mud crabs, these differences in mud crab predation patterns may have resulted in significant differences in oyster survival.

The low predation rates and lack of NCEs in my study suggest that predation is not significantly limiting the recruitment success of oysters in estuarine communities.

However, this may be a consequence of high oyster recruitment as previous experiments have demonstrated that the ability of predators to limit recruitment is facilitated by low recruitment densities (Carrol 1996). Oyster recruitment densities in my study were extremely high (~140 oyster spat/ $0.01m^2$) relative to previous measurements at this experimental site (<50 spat/$.01m^2$, O'Beirn et al. 1995). High recruitment may have resulted because experimental baskets were relatively protected from high sedimentation rates. Oyster larvae often avoid settling in highly sedimented areas (Tamburri et al. 2008) and habitats with increased settlement loads often have less recruitment (Ortega and Sutherland 1992). Thus, high oyster recruitment facilitated by low sedimentation in my study may have overwhelmed mud crab predators. Predation may be a limiting factor under more natural conditions and lower oyster densities. Further, it is possible that increased mud crab predator densities would promote increased oyster mortality. O'Connor et al. (2008) demonstrated that mud crabs at high, but not at low densities, can reduce oyster recruitment. Consequently, variations in predator and prey densities should be examined in order to determine if mud crabs significantly affect oyster recruitment success in the field.

Low predation rates by mud crabs in my study also may have been a consequence of experimental design. As I did not want to restrict oyster recruitment, I chose mesh sizes that only restricted the movement of experimental mud crabs. Consequently, small mud crabs (8-15mm CW) and porcelain crabs (*Petrolistes armatus*) were consistently recruiting and/or immigrating into baskets. Mud crabs are cannibalistic on smaller cohorts (Hill, *personal observations*) and also prey on porcelain crabs in oyster reef habitats (Hollebone and Hay 2008). Thus, mud crab predation rates may have been low

due to feeding preferences for other mobile organisms and previous studies may have overestimated mud crab impacts to oysters by not representing natural multi-species assemblages. Currently, there are few studies that have investigated the feeding preferences of mud crabs among diverse species (but see Hollebone and Hay 2008) and more studies may be required to understand the full impacts of mud crabs in natural reef communities.

Predation by experimental mud crabs also may have been obscured by mud crab immigrants that may have consumed newly settled oyster recruits in baskets. Small immigrant mud crabs were difficult to locate and remove due to their cryptic nature among oyster shells, and I was only able to check and remove immigrating predators every two weeks due to time constraints. This may have led to the high number of scars in both control and blue crab treatments (Fig. 4.3; Fig. 4.4). Consequently, predation rates may appear constant across all treatments because immigrant mud crabs were preying upon oysters in all treatments. In order to accurately address the role of NCEs in oyster reefs and the impacts of predation on oyster recruitment, the immigration of other crab species into experimental habitats must be addressed.

Although mud crabs did not significantly alter the abundance of oysters or scars, there was a significant effect of mud crabs on oyster size class distributions. Larger oysters, >10 and 15mm, were more abundant in controls and treatments of blue crab cues than in either treatment with mud crabs (Fig. 4.6). Although this pattern could result from foraging mud crabs selectively preying on larger oysters, the results of my study do not support this hypothesis. Even after accounting for the total number of scars >5mm (i.e. predation), larger oysters are still less abundant in both the mud crab and mud crab+blue

crab cue treatments suggesting that predation does not cause the shift in oyster size classes (Fig. 4.6). Consequently, this suggests the presence of mud crabs among recruiting oysters are impacting oyster growth.

The lower growth rate of oysters in the presence of mud crabs may be due to oysters reducing their filtering rate and/or time spent open in the presence of crab predators, as seen in other bivalve species (Nakoaka 2000, Smee and Weissburg 2006). Additionally, Johnson and Smee (*unpublished data*) have determined that mud crab chemical cues cause oysters to increase their shell to body mass ratios relative to unexposed oysters. Thus, oysters may be smaller in the presence of mud crabs due to a greater allocation of energy to shell thickness instead of shell growth. By limiting oyster growth, mud crabs increase the time that oysters are vulnerable to a suite of predators which are typically unable to consume larger oyster prey. In addition, these small oysters may be more likely to be overgrown by other oysters or smothered by low rates of sedimentation. Consequently, although mud crabs may not affect absolute recruit abundance, they may contribute to the loss of oyster reefs over longer time scales by increasing their vulnerability to other sources of mortality.

In addition to mud crab predators decreasing the abundance of large oysters, there is also a trend that larger oysters (>10 and >15mm) are more abundant in the presence of only blue crab chemical cues. One possible explanation for this pattern is that excretory wastes from nearby blue crabs are consumed by oysters and contribute to increased oyster growth. Alternatively, the effect of blue crab cues on oyster growth may have been an indirect result of blue crab cues suppressing the abundance of immigrating mud crabs and porcelain crabs. For instance, porcelain crabs decrease oyster growth in natural habitats

and lesser blue crabs (*Callinectes similis*) are a predatory threat to porcelain crabs (Hollebone and Hay 2008). Thus, if porcelain crabs avoid habitats with blue crabs, there may have been an indirect effect on oyster growth. Alternatively, immigrating mud crabs may actively avoid risky blue crab predators that results in less mud crab suppression on oyster growth. In a previous experiment, mud crab predation rates near blue crab predators were substantially lower than within refuge habitats suggesting that mud crabs avoid encounters with blue crabs (Hill and Weissburg Ch. 3). Therefore, it is possible that blue crab cues suppressed the immigration of mud crabs into oyster baskets resulting in less growth suppression than in control baskets. However, fewer mud crab immigrants into blue crab cue treatments also suggests there should be less evidence of predation (i.e. scars) within these baskets. Consequently, I cannot fully support this hypothesis as the amount of predation is not significantly different among treatments (Fig. 4.3). Unfortunately, I also did not record porcelain crab or mud crab abundances in cages over the course of the experiment, thus the impacts of immigrant crab species remains unknown. Future experiments should examine the role of blue crabs on recruitment of multiple crab species and consequences for oyster growth. If blue crabs can significantly and positively impact oyster growth either directly or indirectly, this would further demonstrate that blue crabs can assist the recovery of oyster populations.

In conclusion, the results of my study suggest that predation and NCEs may not be significant enough to limit the survival of recruiting oyster populations. Mud crab predators did not significantly alter the number of oysters surviving or the number of scars. As predation was low, blue crab chemical cues did not have a measured effect on mud crab foraging. These results may be a consequence of high densities of oysters

recruiting into my experiments which effectively swamped mud crab predators. Future studies should address the role of predator and prey densities in limiting oyster recruitment. However, mud crabs significantly impacted the growth of oysters. Although the ultimate cause of this suppression in unknown, growth suppression of oysters will increase the amount of time that oysters are vulnerable to a suite of predators and other abiotic sources of mortality. Thus, even without predation, mud crabs may reduce the number of oysters contributing to reefs over longer time scales.

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